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Review

Symbioses between fungi and bacteria: from mechanisms to impacts on biodiversity

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Symbiotic interactions between fungi and bacteria range from positive to negative. They are ubiquitous in free-living as well as host-associated microbial communities worldwide. Yet, the impact of fungal–bacterial symbioses on the organization and dynamics of microbial communities is uncertain. There are two reasons for this uncertainty: (1) knowledge gaps in the understanding of the genetic mechanisms underpinning fungal–bacterial symbioses and (2) prevailing interpretations of ecological theory that favor antagonistic interactions as drivers stabilizing biological communities despite the existence of models emphasizing contributions of positive interactions. This review synthesizes information on fungal–bacterial symbioses common in the free-living microbial communities of the soil as well as in host-associated polymicrobial biofilms. The interdomain partnerships are considered in the context of the relevant community ecology models, which are discussed critically.

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Introduction

Fungi and bacteria, which collectively contribute about 15% of biomass to the biosphere [1], are potent drivers of global biogeochemical processes, including decomposition of organic matter [2]. The shared saprotrophic lifestyle is expected to promote competition between these organisms. Not surprisingly therefore, fungal–bacterial competition was identified as one of the major forces shaping microbial communities worldwide, including those in the terrestrial topsoil and oceanic habitats [3] as well as in the human gut [4,5]. However, studies of fungal–bacterial relations over the past couple of decades uncovered numerous noncompetitive interactions [6–10] that also could be expected to contribute to structuring and stabilizing of fungal and bacterial communities and to serve as determinants of biodiversity globally [11–17]. Such noncompetitive interactions are known as *symbioses*. They involve the 'living together of dissimilar organisms' and can differ in the balance of fitness costs and benefits experienced by the interacting species, ranging from *mutualisms* through *commensalisms* to *exploitative interactions* [18].

Mutualisms are best viewed as reciprocal exploitations that nonetheless provide net benefits to each participant [19]. Emergent properties of many mutualistic systems contribute to their significance as ecological and evolutionary innovations. For example, the endosymbiotic origin of the eukaryotic cell represented a major evolutionary transition underlying organismal complexity [20]. In *commensalisms*, one partner accrues fitness gains, whereas the other experiences no net effects [21]. At the community level, many of these positive interactions can function as *facilitations* [11] in which the presence of one species alters the environment in a way that enhances growth, survival, or reproduction of a second, spatially, and temporary connected species [22]. In *exploitative symbioses*, such as *parasitism*, one participant benefits at the expense of the other participant [23]. Because of similar fitness outcomes, an argument can be also made for treating *predation* (or fungivory/mycophagy [2]) as part of the symbiotic continuum [23]. Importantly, in fungal–bacterial interactions, the mechanisms underlying exploitative interactions may be difficult to differentiate from the mechanisms behind competition, which yields detrimental outcomes to both partners [23]. Negative outcomes of competition arise due to the depletion of a common resource or reciprocal interference through the synthesis of harmful metabolites [24,25]. Because of the concerns surrounding mechanistic distinctions between exploitative and competitive interactions, I collectively refer to them as *antagonisms*. Importantly, the outcomes of the interactions experienced by populations of the interacting species can vary

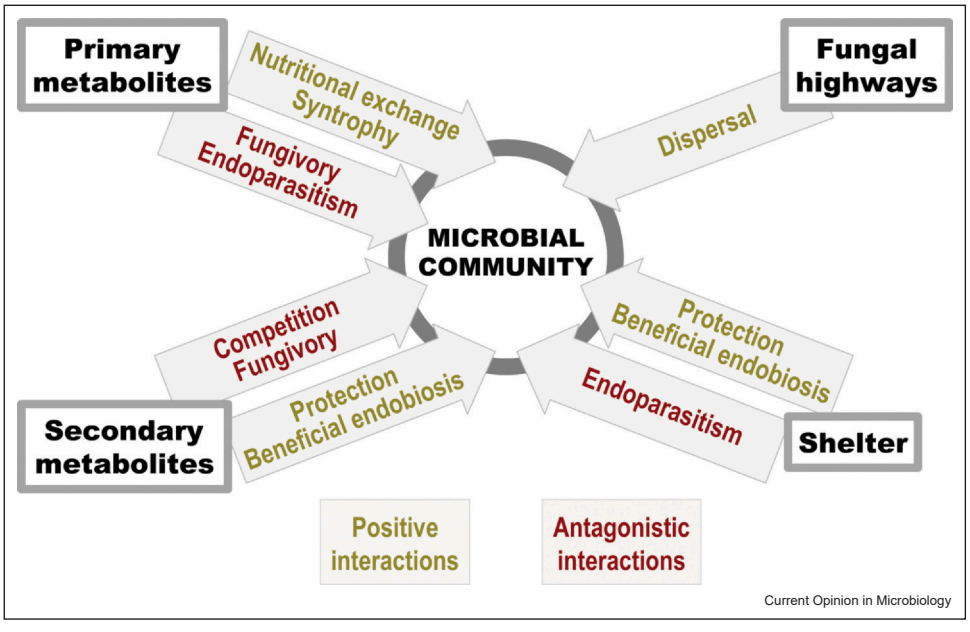
across time and the distribution range inhabited by the symbionts [26]. This pattern is expected to be true for fungal and bacterial partners whether they co-exist in free-living or host-associated microbial communities.

The establishment and maintenance of symbiotic interactions, both mutualisms [27] and antagonisms [28], are influenced by structural aspects of partner body plans and their life cycles, which differ considerably between bacteria and fungi. In particular, bacterial life cycles involve solitary *planktonic cells* that give rise to *biofilms*, which are aggregates of matrix-bound, socially interacting, and highly integrated cells [29,30]. Biofilm-bound cells are metabolically distinct from planktonic cells, a feature that can endow biofilms with emergent properties, such as increased antibiotic resistance. Overall, up to 80% of prokaryotic cells on Earth are estimated to reside in biofilms [31]. In contrast, growth forms of terrestrial fungi include *hyphae* and/or *yeasts* [32]. While the body plans of bacteria and fungi are dissimilar, some yeasts and animal-associated fungi share with bacteria the ability to form biofilms [33]. Moreover, mixed fungal–bacterial biofilms are not uncommon in the context of human disease [34]. Bacteria can also form biofilms on the surface of fungal hyphae [35].

While engaging with their symbiotic partners, fungi and bacteria are also members of microbial communities (Figure 1), that is, collections of interacting populations of different species [36]. Communities have several properties, including diversity, complexity, and stability. Diversity is a measure of the number of different species (species richness) and their relative abundances (species evenness) [37]. Complexity of a community reflects its species richness, how many of its species interact (connectance), and how strongly they interact [38]. Stability indicates the tendency of the system to return to equilibrium after perturbations [39].

Diversity of microbial communities is a key factor in ecosystem functioning worldwide [40,41]. Microbial diversity is also emerging as a determinant of how these systems respond to the perturbations brought about by global change [42]. However, our understanding of the processes governing the organization and dynamics of microbial communities remains limited. While some insights may be gained from conventional community ecology of macro-organisms, it is important to remember that many microbial life cycles are much shorter than those of plants and animals, which affects the rate of change and adaption [43,44]. Macro-organismal community ecology relies on an assortment of models underpinning niche theory and neutral theory [45]. Increasingly, the mechanisms invoked by niche theory (reviewed in Refs. [45] and [46]) and neutral theory [47] are considered to be extreme ends of the same continuum of processes contributing to biological diversity [48,49]. Niche theory ascribes stable coexistence of competing species to their separation along various axes (dimensions) of the ecological niche [45,46], which is defined as multidimensional hypervolume wherein every point corresponds to a state of the abiotic and

Figure 1



Goods and services central to species interactions underlying diversity and stability of microbial communities.

biotic environment that permits the species to exist indefinitely [50]. In contrast, according to neutral theory, all species are equivalent and subject to stochastic processes of immigration, speciation, and extinction [47]. Immigration and speciation are considered stabilizing processes, whereas extinction erodes diversity [51].

Antagonistic interactions are central to many models of niche theory. Competitive exclusion is expected to diminish species diversity, whereas niche partitioning and natural enemies stabilize it [49,51]. Similarly, models developed specifically for microbial communities emphasize antagonisms, suggesting that competition stabilizes the gut microbiome [4], whereas antibiotic interactions maintain diversity [52]. However, there is also a body of ecological theory, suggesting that positive interactions, including mutualisms [12–16], commensalisms [17] as well as community-level facilitative interactions [11], in combination with antagonisms, can stabilize diverse communities of macro-organisms. In particular, the Stress Gradient Hypothesis emphasizes the role of facilitative interactions in harsh physical environments [11], such as arid and semi-arid habitats [53]. Importantly, such unfavorable environments are expected to proliferate worldwide due to global change [54].

To foster creation of experimental strategies needed to elucidate the role of symbiotic interactions in organization and dynamics of microbial communities, I synthesize relevant information about fungal–bacterial symbioses studied in reductionist pairwise settings [55], place them in a broader ecological context, and point out key knowledge gaps. I consider symbioses spanning a range of fitness outcomes, from antagonisms to mutualisms, including partnerships in which *endosymbiotic/endobiotic bacteria (EB)* inhabit fungal cells as either highly adapted [9] or ephemeral [56] symbionts. In my discussion of antagonisms, I focus on fungal *innate immunity defenses* [57], partner *chemical weapons* [58–60], and bacterial *secreted effectors* [61]. I also examine positive interactions in free-living communities of the fungal *hyphosphere* [62] and along *fungal highways* [63] as well as *protective symbioses* [64]. Finally, I discuss host-associated *polymicrobial biofilms* [30].

In nature, fungal–bacterial symbioses are part of a vast network of positive and negative interactions among the microbiota associated with the soil as well as with plant and animal hosts, which, in turn, contribute to the functioning of ecosystems worldwide [65]. These complexities are increasingly appreciated, leading to more holistic approaches for investigating fungal–bacterial relationships. For example, in the soil, fungi and bacteria account for 12 and 7 gigatons of carbon, respectively [1], which creates a vast arena for interactions. Notably, the same soil microbes may engage in multiple partnerships simultaneously or sequentially. The sheer complexity of these multifaceted interactions requires *integrative systems ecology strategies* [66] to infer their impacts on microbial biodiversity at large and to test theoretical predictions, suggesting that antagonisms are not the only interactions contributing to stability of complex microbial communities. In particular, to examine whether positive interactions, such as mutualisms and commensalisms, have a role in structuring of microbial communities, I argue for a two-prong approach that pairs (1) bottom-up reductionist explorations of genetic mechanisms underpinning fungal–bacterial symbioses to identify their molecular mechanisms with (2) top-down dynamic modeling of microbial communities informed by assessments of taxonomic diversity and multiomics profiling to detect the presence of molecular signatures of symbioses.

Antagonisms

Innate immunity

Fungal innate immunity against bacterial antagonists has recently emerged as a theme complementary to fungal–bacterial chemical warfare [57]. The soil mold *Rhizopus microsporus* (subphylum Mucoromycotina) is becoming established as a model to study innate immunity in fungi [67]. Mucoromycotina are ubiquitous soil saprotrophs, postharvest plant pathogens, and opportunistic pathogens of humans [68]. The dearth of genes involved in the synthesis of conventional secondary metabolites [68], which are considered to be important instruments of antibacterial warfare [3] and are common in higher fungi (Dikarya) [60], makes Mucoromycotina an ideal target to probe for nonchemical responses to bacteria perceived as antagonists. Examples of such bacteria include *Mycetohabitans* spp. (Burkholderiales, Betaproteobacteria), which are cultivable mutualistic EB inhabiting host genotypes of *R. microsporus* [69]. The similarities between defensive reactions of *R. microsporus* and innate immunity responses of animals and plants inspired a hypothesis that fungi harbor innate immunity systems with components analogous to the convergently evolved immune mechanisms of animals and plants [67].

Animal and plant innate immune systems consist of surveillance, signal transduction, and response modules [70]. The surveillance modules include cell-surface pattern recognition receptors (PRRs) and cytosolic nucleotide oligomerization domain (NOD)-like receptors (NLRs). PRRs and animal NLRs sense structural components of microbial cells referred to as microbe-associated molecular patterns (MAMPs), such as peptidoglycan, flagellin, lipopolysaccharide, and beta-glucans, as well as damage-associated molecular patterns of host origin. In contrast, plant NLRs interact only with microbial effectors (virulence factors), which are proteins produced specifically to manipulate the host.

While the architecture of fungal surveillance systems remains to be thoroughly investigated, certain patterns begin to emerge. For example, most fungal genomes, with the exceptions of Mucoromycotina and Saccharomycotina, encode large and variable repertoires of up to 200 NLR-like proteins [71]. Some of these NLR-like proteins are involved in self-/nonself-recognition during conspecific fusions of hyphae [72]. However, their role in fungal innate immunity is unclear. In addition, given the diversity of NLR-like proteins in fungi, it is surprising that they do not contain leucine-rich repeat (LRR) motifs important for microbial ligand perception and commonly present in animal and plant receptors [73]. Instead, in fungi, LRR motifs reside in multidomain proteins with the adenylyl cyclase functionality [74]. Fungal adenylyl cyclase proteins are involved in sensing, integrating, and transducing information on a variety of signals, including bacterial peptidoglycan [75]. Fungi are also capable of sensing other MAMPs, such as flagellin and lipooligosaccharide [76]. However, the identity of the receptors involved in the perception of these MAMPs is yet unknown and represents a major knowledge gap in reconstructing immune responses of fungi.

In animals and plants, microbial signals are transduced to activate response modules converging on (1) the release of reactive oxygen species (ROS) [77,78], (2) biosynthesis of antimicrobial peptides [79], which are small peptides that disrupt bacterial cell envelopes, and (3) initiation of regulated cell death (RCD) that eliminates the proliferative niche of invading microbes [70]. Like other elements of innate immunity, fungal response modules remain to be characterized. However, similarities to animal and plant reactions have already been observed. For example, *R. microsporus* confronted by the *Mycetohabitans* bacteria perceived as antagonists produces ROS [67], whereas coprophilous *Coprinopsis cinerea* synthesizes defensin and lysozyme antimicrobial peptides in response to *Bacillus subtilis* and *Escherichia coli* [80]. Notably, it remains to be tested whether fungi deploy immune RCD to eliminate bacterial intruders.

Fungivory

It is expected, although has not been tested explicitly, that fungi engage innate immune mechanisms in response to predation by fungivorous bacteria. Fungivory (also known as mycophagy) is manifested as the ability of bacteria to grow at the expense of their fungal prey [2]. The prevalence of this lifestyle among various bacterial lineages is uncertain [81]. The notable examples of bacterial fungivores include *Collimonas* spp. [82], *Burkholderia gladioli* [83,84], and *Pseudomonas tolaasi* [85]. These bacteria, in addition to proteases, secrete enzymes degrading fungal cell walls, including chitinases [86], as well as antifungal secondary metabolites, such as the membrane pore-forming lipopeptide tolaasin [85].

Another mode of killing the fungal prey is deployed by *Pseudomonas syringae*, which is able to induce RCD in the ascomycete *Neurospora crassa* by expressing a protein with homology to one of the determinants of vegetative/heterokaryon incompatibility in this fungus [87]. In general, self-/nonself-recognition during conspecific fusions of hyphae in ascomycetes is mediated by the *het* loci, with co-expression of incompatible alleles at the same locus or incompatible alleles at different loci leading to RCD of the heterokaryotic portions of the mycelium [88]. In the case of interactions with *Pseudomonas syringae*, the bacterial protein highjacks this incompatibility reaction, leading to suicidal death of fungal hyphae [87].

A glimpse of the interplay between antibacterial and vegetative incompatibility responses comes from the coprophilous ascomycete *Podospora anserina*. In *P. anserina*, transcriptional profiling revealed that most functions upregulated in response to antagonistic *Serratia marcescens* favor prosurvival autophagy, whereas functions upregulated during vegetatively incompatible interactions lead to RCD, which is mediated by the NLR-like proteins [89]. Interestingly, the ability of *S. marcescens* to induce death in yeast *Saccharomyces cerevisiae* and *Candida* spp. is related to specific type VI secretion system (T6SS) effectors [90] that cause loss of plasma membrane potential and disrupt nutrient uptake/metabolism in fungal cells. Clearly, further work is needed to evaluate the links between antibacterial defenses and the mechanisms of self-/nonself-recognition. The ubiquity of NLR-like genes in the genomes of asco- and basidiomycetes, together with the significance of NLRs in innate immunity of plants and animals, imply functional significance of these molecules in fungi. However, their specific roles in symbiotic interactions remain to be investigated.

Chemical warfare

Despite the unquestionable contribution of microbe-derived antibiotics to antimicrobial therapies [91], the hypothesis that secondary metabolites are primary instruments of chemical warfare underlying fungal–bacterial antagonisms has been repeatedly challenged [92]. These challenges are based on observations that antimicrobials (1) act as signaling molecules in bacteria [93], (2) protect fungi against insect grazers [94], and (3) are instrumental in bacterial colonization of fungal cells [95–98]. Therefore, caution is needed to avoid overinterpreting the role of antimicrobial secondary metabolites in fungal–bacterial antagonisms. Such overinterpretations could arise readily because conventional genes involved in secondary metabolite biosynthesis and protection against them can be easily identified based on homology with known genes. As a consequence, it is increasingly more achievable for

ecological studies to include information on antimicrobials and the means of neutralizing them [3].

In addition to producing secondary metabolites, fungi secrete small proteins able to suppress bacterial competitors [99]. These secreted antimicrobial proteins resemble effectors used by fungal pathogens and mutualists to manipulate plant hosts and are thought to have evolved in ancestors of extant fungi before the emergence of land plants [61].

Overall, fungal perception of and responses to antagonistic bacteria are far from understood. It is conceivable that, at least in some fungi, these responses involve production of defensive secondary metabolites and effectors in addition to prototypical innate immune responses involving the biosynthesis of ROS and antimicrobial peptides. Therefore, gathering observations permitting integration of these patterns into a cohesive predictive framework is of utmost importance for understanding mechanistic underpinnings of fungal–bacterial antagonisms.

Positive interactions

Hyphosphere

The sheer multitude of failed attempts to obtain bacteria-free cultures of soil fungi despite the use of antibiotics in isolation procedures attest to the intimacy of fungal–bacterial interactions in the soil [100–102]. Such interactions often involve bacteria residing in the fungal hyphosphere, defined as the volume of substrate under the influence of fungal hyphae [62]. For example, species of the genus *Burkholderia* show a significant pattern of co-occurrence with soil fungi, including plant pathogens as well as ancient mutualists of plants, arbuscular mycorrhizal fungi (AMF, subphylum Glomeromycotina) [103]. Unlike saprotrophic fungi, which derive their energy from decomposition of organic matter, AMF rely on plants for carbon sources [104]. As a consequence, AMF are shielded from antagonistic competition with bacterial decomposers of organic matter and are thus more readily able to establish positive interactions [105]. There is ample evidence that bacteria in the AMF hyphosphere benefit from carbon-rich fungal exudates [106,107]. These compounds select for bacterial communities distinct from those present in the surrounding soil [108–110]. In particular, the AMF hyphosphere microbiomes are enriched in phosphate-solubilizing bacteria, which mineralize organic phosphorus, such as phytate, into inorganic phosphorus thus making it available to AMF [109]. Because of the ubiquity of AMF associations with plants and their paramount role in plant phosphorus nutrition, the molecular mechanisms underlying facilitative mineralization of phosphorus by hyphosphere bacteria deserve attention. AMF hyphae also serve as thoroughfares permitting translocation of phosphate-solubilizing bacteria, such as *Rahnella aquatilis* (Enterobacteriales, Gammaproteobacteria), to patches of soil enriched in organic phosphorus [111].

Fungal highways

Bacterial travel along hyphal highways [63] may be one of the most common positive interactions between bacteria and fungi in the soil [112,113] and other unsaturated habitats [114]. However, not all bacteria [115] or fungi [116] are able to engage in this mode of bacterial transportation. While bacteria have evolved an array of diverse motility mechanisms, they all require either aqueous media or a thin layer of liquid on solid surfaces [117]. Therefore, bacterial traffic across air-filled porous substrates, such as soil [112,113] or cheese rinds [114], may be impeded by the absence of a continuous film of liquid. In contrast, such substrates can be readily penetrated by expanding mycelial networks composed of hydrophilic hyphae [118], which themselves are covered by an aqueous layer permissive to bacterial motility [63,116]. Such highways are used by bacteria exhibiting intrinsic flagellar motility to speedily reach chemo-attractive targets [111,115,119,120]. Active travelers may also enable transportation of hitchhikers, which are unable to journey along the highways on their own [121]. Examples of bacterial travelers include bacteria preying on other bacteria, such as *Bdellovibrio bacteriovorus*, utilizing fungal hyphae to reach sites harboring suitable prey [122] or plant-associated bacteria dispersing along the hyphae of plant-associated fungi, such as rhizobia that use the highways formed by the endophytic fungus *Phomopsis liquidambaris* to connect with and nodulate their shared legume hosts [123]. At least some bacterial travelers compensate their fungal transit facilitators with goods or services, which turns these otherwise commensal interactions into mutualisms. For example, *Bacillus subtilis* rewards *Aspergillus nidulans* with thiamine [124]. *Rahnella aquatilis* makes soil phosphorus available to AMF and their plant hosts [111]. *Burkholderia terrae* protects the soil basidiomycete *Lyophyllum* sp. against negative effects of *Pseudomonas fluorescens* and the fungicide cycloheximide [116].

Protective/defensive symbioses

Not all protective services involve compensating for passage along fungal highways by protecting transportation providers against bacterial toxins [116]. For example, the membrane pore-forming tolaasin toxin produced by the fungivorous *P. tolaasi*, which damages fruiting bodies of commercially grown *Agaricus bisporus* (white button mushroom) and *Pleurotus ostreatus* (oyster mushroom) [125], is broken down by the *Mycetocola* spp. bacteria (Micrococcales, Actinomycetota) co-occurring with bacterial fungivores on basidiocarps [126]. Similarly, species of *Paraburkholderia* protect representatives of *Aspergillus* and *Penicillium* molds against bacterial redox-active phenazines by forming phenazine-

sequestering aggregates within fungal mycelia, while the fungus maintains conditions that limit phenazine toxicity by promoting an anoxic and highly reducing environment [127]. Another way bacteria engage in protective symbioses is by synthesizing secondary metabolites essential for defending fungi against predation by fungivorous amoebae [128,129] and nematodes [129,130], a pattern exemplified by several EB discussed in the later sections of this paper. Remarkably, fungi are not only the recipients but also providers of protection. For example, nematode-trapping fungi defend bacteria from predation by bacterivorous nematodes after bacteria in distress release urea, which mobilizes fungi to produce nematode killing traps [131].

Interactions in polymicrobial biofilms

Many free-living soil fungi and bacteria are recruited to host-associated habitats where they often engage in novel interactions involving polymicrobial biofilms. Biofilms are widely accepted as a major form of prokaryotic life [31]. However, biofilms uniting fungi and bacteria seem to be limited to host-associated environments, such as animal respiratory and gastrointestinal tract, including the herbivore rumen [132]. A lot of attention is being received by biofilms linked to medically important polymicrobial infections of humans because the biofilm-bound partners often behave differently from their free-living counterparts [34]. For example, *Candida albicans* and *Pseudomonas aeruginosa* act antagonistically outside biofilms [133] but in mixed biofilms operate synergistically [134]. Such synergistic interactions not only contribute to the enhancement of structural complexity, as observed in the *Candida albicans–Staphylococcus aureus* biofilms, but also to increased tolerance against antibiotics [134], which may contribute to poor therapeutic outcomes.

Biofilms are also abundantly present in the gastrointestinal tract where they often form on food particles as well as on mucus or epithelia [135]. One of the most prominent examples of fungal–bacterial biofilms comes from the rumen of herbivorous mammals. The rumen is an anaerobic environment lacking oxygen as a terminal electron acceptor, which is a setting favorable to syntrophy, that is, obligate metabolic cooperation beneficial for all partners involved [136]. The syntrophy practiced by the rumen microbiota is methanogenic fermentation of plant biomass. *Nota bene*, methanogenic fermentation is central to livestock farming, which constitutes a significant source of anthropogenic methane emissions fueling global change [137].

In the rumen, the primary fermenters, including the early divergent Neocallimastigomycota fungi [138], decompose lignocellulosic plant material ingested by their herbivore hosts [139]. In the absence of mitochondria mediating oxidative phosphorylation, Neocallimastigomycota are obligate anaerobes [140]. To meet their energy needs without oxygen, they rely on hydrogenosomes, organelles that generate molecular hydrogen. However, the accumulation of hydrogen causes feedback inhibition of the fermentation process unless hydrogen molecules are scavenged by syntrophic methanogenic archaea to produce methane [141]. While the syntrophic partners in the rumen are highly coadapted, theoretical modeling predicts that syntrophies can also emerge spontaneously [142] as products of ecological fitting [143]. It is, therefore, intriguing whether Neocallimastigomycota, which are able to survive outside the mammalian hosts [144] and whose DNA sequences have been reported from anaerobic environments [145], can engage in spontaneously formed syntrophic interactions outside of the rumen, for example, in anoxic sediments.

The syntrophic interactions between anaerobic fermenters and archaeal methanogens are widely appreciated as mutualisms. However, fermentative bacteria and fungi may engage in antagonistic interactions with each other [146]. Such antagonisms between bacteria and fungi coexisting in the gastrointestinal tract have been implicated as a selective force driving horizontal transfer of genes from bacteria to fungi [147,148]. Horizontal gene transfer is commonly recognized as a mechanism for genome diversification and the acquisition of novel functions [149], with the gut milieu serving as a hot bed of such acquisitions [150]. Accordingly, the genomes of Neocallimastigomycota show evidence of horizontally acquired bacterial genes encoding antibiotic resistance enzymes [147]. Similarly, in the amphibian gut symbiont *Basidiobolus* (phylum Zoopagomycota), the genes encoding toxins, surfactants, and antibiotics appear to be of bacterial origin [148].

Altogether, host-associated fungal–bacterial biofilms show benefits of positive interactions, including synergy, syntrophy, and transfer of genetic material among the partners. It is worth noting that, in contrast, interactions among participants of exclusively prokaryotic polymicrobial biofilms are often antagonistic [30].

Endosymbiotic interactions

Even though fungi evolved in a world teeming with bacteria, the interactions of the early fungi with bacteria remain uncertain. Living fossils, such as AMF, suggest that fungi harbored EB as early as 400 mya, as inferred from the heritable mutualism between the AMF family Gigasporaceae and '*Candidatus* Glomeribacter gigasporarum' (Burkholderiales, Betaproteobacteria) [151]. AMF are credited with a pivotal role in the Siluro-Devonian terrestrialization of plants [152] and as determinants of plant biodiversity, ecosystem variability, and

productivity worldwide [153]. These inferences are related to the ability of AMF to provision their plant hosts with mineral nutrients translocated from the soil in exchange for plant-assimilated carbon [154]. In turn, AMF rely on '*Candidatus* Glomeribacter gigasporarum' for fine-tuning of their energy metabolism, which supports improved germ tube expansion [155]. However, '*Candidatus* Glomeribacter gigasporarum' is not essential to its fungal partners, even though it is uncultivable [156] and highly adapted to the host cellular environment [157]. Nevertheless, evolutionary longevity of this mutualism, supported by the features of the '*Candidatus* Glomeribacter gigasporarum' genome [157], is a clear indication of the significance of its services to the fungal host. In addition to '*Candidatus* Glomeribacter gigasporarum', many AMF harbor an enigmatic mycoplasma-related endobacterium (MRE) '*Candidatus* Moeniiplasma glomeromycotorum' (Mollicutes, Mycoplasmatota) [158]. The role of this heritable symbiont in the AMF host biology is unknown. However, genomic features of '*Candidatus* Moeniiplasma glomeromycotorum' suggest its metabolic dependence on the host and a parasitic lifestyle [159–161].

While the lifestyle of '*Candidatus* Moeniiplasma glomeromycotorum' remains uncertain, the closely related MRE has been confirmed to be a parasite of Mortierellomycotina [162], yet another lineage of early divergent soil fungi found in plant rhizosphere and roots [68]. In addition to MRE [162], Mortierellomycotina are often colonized by vertically transmitted *Mycoavidus* spp. (Burkholderiales, Betaproteobacteria) [163,164] that, under laboratory conditions, was also shown to inflict a fitness cost on the fungus [165]. However, under natural conditions, *Mycoavidus* can act as a conditional mutualist, protecting its fungal host from fungivorous nematodes [130]. This protective service is linked to the biosynthesis of highly toxic macrolactones, known as necroximes, which also serve as examples of nonantimicrobial secondary metabolites. The soil mold *R. microsporus* also harbors EB that offer chemical protection from fungivory: *Ralstonia pickettii* that shields its hosts from predation by soil amoebae [128] and *Mycetohabitans rhizoxinica* that produces the antimitotic toxin rhizoxin active against amoebae as well as fungivorous nematodes [129].

While both Glomeromycotina and Mortierellomycotina are facultative hosts to EB, their bacterial symbionts lost the ability to grow in isolation from fungi, that is, they are obligately dependent on their fungal partners and lack amenability to culturing. Therefore, studying the mechanisms underlying the establishment of these symbioses is largely impractical. However, fungi also form heritable symbioses in which EB show a considerable degree of coevolution with the hosts but remain cultivable. These associations include the previously mentioned mutualism between *R. microsporus* and *Mycetohabitans* spp., which is emerging as a model for studies of the molecular underpinnings of fungal–bacterial endosymbioses [69].

Conceptual framework

Like with fungal defense responses, in the absence of established models, a conceptual framework for fungal–bacterial endosymbioses can be formulated based on observations from convergently evolved phenomena in animals and plants [166,167]. Notably, these symbiotic animals and plants also rely on immune mechanisms for modulating their interactions with mutualistic microbes, as exemplified by two focal systems: (1) the symbiosis of the Hawaiian bobtail squid *Euprymna scolopes* with *Vibrio fisheri*, whose bioluminescent light camouflages the host [168,169] and (2) legume symbiosis with nitrogen-fixing rhizobia bacteria [167,170].

The process of light organ colonization in juvenile squid involves highly orchestrated interactions between host PRRs and *Vibrio* MAMPs, such as peptidoglycan and lipopolysaccharide [168,169]. In the legume–rhizobium mutualism, the host PRRs also play a critical role in recognizing rhizobial MAMPs and initiating a classical immune response that is rapidly suppressed by signals originating from the interaction between the rhizobial lipo-chitooligosaccharide nodulation (Nod) factor and the legume LysM (lysin motif) receptors, which are most likely derived from ancient immune receptors of chitin [170]. Perception of the Nod factor as well as rhizobial type III secretion system (T3SS) and type IV secretion system (T4SS) effectors triggers plant signaling cascades that lead to accommodation of the symbionts in the nodule, which becomes a site of symbiont-mediated nitrogen fixation [170].

Mechanisms

Because the *Mycetohabitans* spp. mutualists of *R. microsporus* are readily cultivable and can be reintroduced to a previously cured fungal host [69,128], this heritable symbiosis is superficially comparable to the animal and plant models of symbiosis. However, the *Mycetohabitans* EB provision the fungus with secondary metabolites [69,128,171], while controlling its reproductive biology [172,173], which renders the fungus functionally addicted to the symbionts, a circumstance distinctly different from those in the squid–vibrio or the legume–rhizobium symbiosis.

Several important insights about the mechanisms of fungal–bacterial interactions have already emerged from the *R. microsporus–Mycetohabitans* symbiosis. For example, analogously to legume–rhizobium symbiosis, the *R. microsporus* host responds to *Mycetohabitans* colonization with a subsequent dampening of an initial ROS burst, which is a significant component of the immune response in the nonhost

fungal genotype [67]. The analogy extends to the *Rhizopus* host accommodating the symbiont by altering its own lipid metabolism [174]. Also akin to the rhizobial symbiosis factors, several *Mycetohabitans* T3SS [175,176] and type II secretion system (T2SS) effectors, including chitinolytic enzymes [177], are essential for colonization of the previously cured host as well as symbiosis maintenance [176,178]. Finally, recent evidence points to the critical role of the bacterial cyclopeptide habitasporin in colonization of fungal hyphae [95]. However, it is not clear whether habitasporin is a functional equivalent of the rhizobial Nod factor responsible for dampening the immune responses in legumes to facilitate nodule development and symbiotic nitrogen fixation [167].

Bacterial secondary metabolites also have roles in symbioses in which bacteria are ephemeral associates of fungi. For example, cyclic lipopeptides produced by diverse bacteria, both Gram-negative (*Pseudomonas aeruginosa* [97], *Ralstonia solanacearum* [98]) and Gram-positive (*Bacillus subtilis* [96] and *Bacillus velezensis* [97]), induce formation of thick-walled perennating chlamydospores in a variety of fungal species [96,98]. Invasion of chlamydospores by lipopeptide-producing bacteria as well as by chemically naïve hitchhikers creates a proliferative niche for ephemeral associates of fungi, facilitating bacterial persistence through the periods of unfavorable environmental conditions [97,98].

The ease of bacterial internalization into fungal cells may explain the ubiquity of these ephemeral EB reported in fungi across various habitats [128,179–191]. At least some of these endosymbionts appear to improve the fitness of their fungal hosts [181,184,191]. For example, *Bacillus* sp. [181] and *Klebsiella michiganensis* [192], EB of the maize pathogenic fungus *Ustilago maydis*, as well as *Pseudomonas stutzeri* associated with *Rhodotorula mucilaginosa*, a growth-promoting plant endophyte, fix nitrogen for their fungal hosts [193]. Similarly, a horizontally transmitted bacterium *Luteibacter* sp. appears to enhance the biosynthesis of the phytohormone indole-3-acetic acid (IAA) by its fungal host *Pestalotiopsis* sp. (Xylariales) [194]. *Pestalotiopsis* sp. is a foliar endophyte of oriental thuja (*Platycladus orientalis*) and may benefit from the IAA-mediated improvement of the host plant fitness [194] through the mechanism of partner fidelity feedback [195].

Altogether, the mechanisms underlying the formation of symbioses in which bacteria dwell in fungal cells are beginning to emerge. Several bacterial symbiosis factors have been already identified. These include T2SS and T3SS effectors [175–177] as well as various secondary metabolites [95–98]. In contrast, fungal mechanisms underpinning bacterial accommodation remain poorly understood. In the early divergent Mucoromycotina fungi, the mutualism between *R. microsporus* and *Mycetohabitans* constitutes a promising model system for such studies [67,173,174]. Importantly, Mucoromycotina appear to lack canonical NLR-like proteins [71] and display a limited repertoire of secondary metabolites [67], which are both common in Dikarya. The absence of these molecules may indicate that the innate immunity/symbiont accommodation mechanisms of Mucoromycotina are either ancestral or highly specialized and differentiated from those of asco- and basidiomycetes.

Interactions underpinning stable and diverse microbial communities

Many verbal arguments [45,46] and mathematical models [4,39,52] favor antagonistic interactions as drivers of diversity and stability in biological communities. For example, antagonisms play a central role in explanations of stable species coexistence offered by the models of niche theory [48,49]. Specifically, the process of niche partitioning along the trophic and natural enemy axes, which involve antagonistic interactions with consumers, prey, pests, and pathogens, is expected to promote diversity. However, there are also models that emphasize the role of positive interactions [11–14,17]. Both trophic and natural enemy relations can be further modified by positive interactions with mutualists provisioning metabolites or protective services, thus altering ecological niches of interacting species and affecting community stability [12].

Formalizing such arguments through dynamic modeling of biological communities as ecological networks consisting of nodes (representing taxa) and links/edges (denoting interactions) yielded profound quantitative insights. For example, in models in which species pairs interact exclusively either in predator–prey, mutualistic or competitive fashion, predator–prey exploitative interactions stabilize the network, whereas mutualistic and competitive interactions destabilize the networks [39]. Such models were refined by Mougi and Kondoh by allowing individual species to engage in both exploitative and mutualistic interactions in varying proportions [13]. The results indicated that small contributions of mutualistic interactions destabilize the network. However, when a mutualistic interaction's contribution increases to a moderate level, the network becomes more stable. The applicability of this outcome to the mammalian gut microbiome was challenged by Coyte et al. on the ground that, unlike macro-organisms, microbes do not have set capacities for different types of interactions but rather their interactions morph fluidly from antagonisms to mutualisms and vice versa [4]. Allowing for such fluid transitions has the potent consequence of eliminating the stabilizing influence of mutualisms in predominantly competitive networks. Importantly, this modeling outcome was empirically validated by observations from Stein et al., suggesting that mutualisms account for only ~15% of interactions in the human gut microbiome [4,5].

The argument of Coyte et al. that microbial interaction budgets are fluid is certainly valid for some fungi and bacteria [4]. However, as demonstrated in the preceding discussion, lifestyles and interaction types of many fungi are constrained by their evolutionary history. For example, due to their biotrophic dependance on plants, AMF do not compete for carbon with saprotrophic bacteria or fungi [154]. Similarly, rumen-associated Neocallimastigomycota rely on syntrophy with methanogenic archaea for uninterrupted decomposition of lignocellulosic plant material [138]. Considering these systems, positive interactions should not be dismissed as a force stabilizing microbial communities comprising such fungi, including in soil and in the herbivore rumen.

Another setting expected to be conducive to the development of stabilizing positive interactions are microbial communities under harsh physical conditions. This expectation is related to the Stress Gradient Hypothesis formulated for and well supported in plant communities [11,53]. The Stress Gradient Hypothesis is also gaining support in plant-associated microbial communities [196,197]. In particular, observations along the stress gradients of decreasing nutrient and water availability in the Florida scrub ecosystem indicated that relative abundance of microbial mutualists of plants was positively correlated with stress increase, whereas the prevalence of plant fungal pathogens correlated negatively with stress [197]. In addition to these observations, it could be hypothesized that bacterial use of fungal highways increases with reduced soil water availability and will become more pronounced worldwide as the existing trend toward desertification is expected to continue and intensify under global change [54].

Ultimately, the neglect of positive interactions in microbial community ecology may be related to the notion that chemical warfare is unique to microbes and therefore able to account for microbial diversity, which is orders of magnitude higher than the diversity of known macro-organismal communities [52]. Accordingly, the instruments of fungal–bacterial warfare in the form of antibiotic and resistance genes have been recently used as interaction proxies in the study testing whether biotic interactions play a role in shaping microbial communities in the topsoil and ocean habitats worldwide [3]. Not unexpectedly, without an avenue for detecting positive interactions, this approach yielded overwhelming evidence for the importance of fungal–bacterial antagonisms in microbial diversity globally.

Closing knowledge gaps and integrating levels of organization

The preceding discussion of fungal–bacterial symbioses illuminates the vast knowledge gaps in basic understanding of the mechanisms behind both negative and positive interactions. The role of secondary metabolites in fungal–bacterial antagonisms requires critical evaluation, as not all these compounds are instruments of chemical warfare. Some secondary metabolites are regulatory molecules or signals for mutualistic partners [93,95–98] (Figure 1). Similarly, bacterial effectors (virulence factors), such as chitinases [86], known to manipulate eukaryotic hosts, including fungi, are increasingly being rediscovered as symbiosis factors in fungal–bacterial mutualisms [177]. Besides the peptidoglycan-sensing adenylyl cyclase [75], fungal PRRs are virtually unknown and yet may have roles in both antagonistic and positive interactions. Therefore, it is critical to identify the fungal PRRs involved in sensing both antagonistic and mutualistic bacteria. Fungal responses to bacteria differ depending on whether the symbionts are perceived as antagonists versus mutualists. The efforts to characterize these interactions are underway and a centralized resource of a publicly accessible database for fungal–bacterial interactions research has been recently unveiled [198]. Although many studies have been limited to cataloging transcriptional dialogs between the partners, a lot of functional data are already available [43,44,67,76,80,87,89,90,174,175,177,199–201]. Observations concerning mechanisms underlying how different groups of fungi (Mucoromycotina vs Dikarya) interact with antagonistic versus mutualistic prokaryotes need to be integrated into a common predictive framework. Such a framework is already being formulated by merging information from an array of fungal–bacterial symbioses with the principles that govern microbial interactions of other eukaryotes, including animals and plants. Once available, this framework will become a treasure trove of molecular hallmarks typifying various fungal–bacterial interactions. It bears noting that distinguishing between the mechanisms underlying positive versus negative interactions is not trivial (Figure 1). Similarly, uncovering the underpinnings of facilitative interactions in the soil (hyphosphere and fungal highways) is bound to be challenging. Nevertheless, recognizing the significance of these interactions in diversity and stability of microbial communities is a pivotal step toward assessing their impacts.

The simplest way of representing interactions among species in the community is through reconstructing a species co-occurrence network and quantifying its structure [38]. Examination of networks representing microbial communities has already generated important insights concerning structural properties of these communities [202]. For example, network analysis of plant-associated microbial communities along the gradients of decreasing nutrient and water availability revealed that microbial diversity as well as network modularity (the level of compartmentalization) and cohesion (connectivity of the entire sampled community) declined with stress [197]. These changes in network properties were interpreted as increased destabilization of the soil microbiome with escalating stress. Such structural

analyses of ecological networks are useful and popular [38]. However, their predictive value is limited, and they are gradually supplanted by dynamic models [203]. Dynamic models have been widely used to make theoretical predictions about conditions stabilizing ecological networks in general [4,13,14,17,39]. Such models can also be parameterized with empirical data, including taxon abundance and multiomics data, to infer microbial interactions as well as network stability and possible ways to manipulate the networks toward desired states [203]. Thus far, such outcomes have been achievable only for small communities comprised of microbiota with well-characterized metabolic profiles [204]. However, with the rapid pace of global change, there is a pressing need to predict the consequences of imminent shifts in the diversity of fungal–bacterial communities worldwide. Such predictions require a thorough understanding of the forces that stabilize these communities, including various symbiotic interactions discussed here and their mechanistic underpinnings.

Conclusions

Fungal–bacterial symbioses are diverse and ubiquitous in free-living and host-associated microbial communities worldwide (Figure 1). They are also critical to the functioning of terrestrial ecosystems, especially under the conditions of global change. This review identifies two considerations that need to be addressed in order to understand the role of symbiotic interactions in microbial communities. First, many important symbioses are poorly understood at the mechanistic level. This lack of knowledge impedes the use of multiomics tools to detect them in sampled environments. Second, an influential body of ecological theory favors antagonistic interactions as forces stabilizing biological communities despite the existence of models emphasizing the role of positive interactions. Thus, there is an urgent need to close knowledge gaps surrounding mechanistic underpinnings of fungal–bacterial symbioses and to refine ecological theory by developing models inclusive of various types of interactions. Once such advances are made, constructing dynamic models will allow for inferences about the role fungal–bacterial interactions will play in stabilizing microbial communities in the face of global change.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- • of special interest
- •• of outstanding interest

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