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Cross-feeding in the gut microbiome: Ecology and Mechanisms

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Abstract

Microbial communities are shaped by positive and negative interactions ranging from competition to mutualism. In the context of the mammalian gut and its microbial inhabitants, the integrated output of the community has important impacts on host health. Cross-feeding, the sharing of metabolites between different microbes, has emergent roles in establishing communities of gut commensals that are stable, resistant to invasion, and resilient to external perturbation. In this review, we first explore the ecological and evolutionary implications of cross-feeding as a cooperative interaction. We then survey mechanisms of cross-feeding across trophic levels, from primary fermenters to H₂ consumers that scavenge the final metabolic outputs of the trophic network. We extend this analysis to also include amino acid, vitamin and cofactor cross-feeding. Throughout, we highlight evidence for the impact of these interactions on each species' fitness as well as host health. Understanding cross-feeding illuminates an important aspect of microbe-microbe and host-microbe interactions that establishes and shapes our gut communities.

Introduction

Microorganisms in nature do not typically exist in monoculture under constant conditions, but instead form complex communities that withstand fluctuating environments. The interactions between these microbes produce synergistic responses that often cannot be predicted by studying individual species on their own; competitive and antagonistic interactions restrict the growth of some members, while the combined metabolic activity of the community buffers against perturbation during nutrient stress. Many of these ecological dynamics are mediated by diffusible metabolites which can act as nutrient sources, inhibitory compounds, or signalling molecules.

The human body is an important habitat for microbial colonization, hosting ~100 trillion microbial symbionts¹. Of the body sites that are colonized, microorganisms inhabiting the intestinal tract, or gut microbiome, are by far the most abundant and diverse, and include bacteria alongside lesser studied fungi, viruses, and other microbes. The gut microbiome is

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Competing Interests

The authors declare no competing interests.

home to more than 1000 different bacterial species at the population level and at least 150 species in an individual². These microbes have far-ranging impacts on health, determined by their encoded metabolic capacity and interactions with the host.

Cross-feeding is the exchange of metabolites as energy and nutrients among different species or strains of microorganisms³. In the context of the gut microbiome, metabolite sharing can also occur between host and microbe, though we will focus on microbe-microbe interactions in this review. Microbial cross-feeding is not unique to the gut microbiome and occurs in many, if not all, natural communities. Both anecdotal evidence and genome-scale analysis highlight the breadth of cross-feeding across microbial communities. For example, mapping interspecies metabolic exchanges predicted from genome sequences across 800 host-associated and free-living microbial communities identified co-occurring subcommunities of species with a significantly higher-than-chance potential for cross-feeding⁴. The observed high frequency of cross-feeding is consistent with division of labour in microbial communities, a common theme among all biological systems. Furthermore, ill-defined metabolic requirements that are provided through cross-feeding in a community setting may explain our difficulty culturing various microbes axenically⁵.

In addition to its pervasive presence, understanding cross-feeding in the context of the gut is important to understand the microbiome's impact on host health. Cross-feeding is an important mechanism shaping community composition, response to perturbation, the community's integrated metabolome, and together their emergent interactions that affect the host. In this review, we will describe the ecological forces and consequences of cross-feeding interactions, then explore cross-feeding mechanisms of different nutrients in the gut microbiome and their impact on host health.

Fitness impacts of cross-feeding

To understand the significance of cross-feeding in the gut microbiome, we first consider the role it plays in shaping community composition, stability, and resilience. Members of microbial communities, including those in the gut microbiome, interact with and impact the growth of other microbes in ways that can be classified in terms of the fitness of members involved (Figure 1)⁶. On the negative side, competition (−/−) lowers the fitness of both involved, while amensalism (−/0) harms one species while the other is unaffected. On the positive side, exploitation/parasitism/predation (+/−) benefits one member to the detriment of the other, commensalism (+/0) benefits one member and is neutral to the other, and mutualism (+/+) benefits both members. These interactions can occur both intra- and interspecifically, though cross-feeding is usually considered to be an interspecific interaction.

Cross-feeding in the gut microbiome can be exploitative, commensalistic or mutualistic (Figure 1a-d). Exploitation can occur when a consumer benefits from a substrate produced by a partner organism while also changing the environment in a way that harms this producer, such as by secreting a toxic waste product (Figure 1d). Alternatively, commensalistic cross-feeding results when one microbe feeds on metabolites produced by another microbe with no impact to the latter (Figure 1c).

Cross-feeding interactions that are mutualistic occur when two species each feed on a metabolite produced by the other (Figure 1a), or when one microbe feeds on a metabolite from another and also modifies the environment in a way that benefits the producer (Figure 1b). These cooperative interactions are termed syntrophy, classically defined as a form of “obligately mutualistic metabolism” where together the partners exploit substrates that neither could metabolize alone under a given set of conditions⁷. The quintessential example of syntrophy is interspecies H₂ transfer (discussed in detail below). Other examples of syntrophy include reciprocal feeding on metabolites produced by partner microbes. For example, in the *Drosophila melanogaster* gut, *Acetobacter pomorum* feeds on lactate produced by *Lactobacillus plantarum* and in turn produces essential amino acids required for *L. plantarum* growth (discussed further in the amino acid cross-feeding section)⁸.

The community stability paradox

Understanding the extent of mutualistic cross-feeding interactions in the gut microbiome is fundamental to explaining some of its core characteristics: extraordinary diversity of species and remarkable stability in maintaining the same taxa over the course of years to decades⁹. In ecological theory, true syntrophic interactions lead to interacting species that “boom and bust” together, resulting in unstable, low diversity communities susceptible to invasion and characteristic of dysbiosis. Furthermore, cooperative interactions involving public goods, defined as compounds or functions released into the extracellular environment for a collective benefit¹⁰, are susceptible to promoting cheaters that utilize these compounds but do not contribute to their production and threaten to collapse cooperative behaviour. For comparison, competitive interactions can stabilize microbial communities by reducing the strength of mutualism and blocking any one species from outcompeting the rest of the community¹¹. Different studies come to different conclusions about the abundance of cooperative cross-feeding in the gut microbiome; while some report that these communities are dominated by cooperative interactions^{3,12,13}, others report that competition is the primary microbial interaction^{14,15}. These discrepancies highlight two key questions: what is the true frequency of cooperative cross-feeding in the gut microbiome, and how do communities remain stable in the presence of cross-feeding interactions?

The answer to these questions likely lies with ecological forces in the gut that reductionist experiments and computational models fail to measure or predict. For example, measuring the frequency of cross-feeding interactions using pairwise measurements between species fails to account for higher order interactions with other species, computational models for gene content or metabolic flux often fail to consider gene regulation, and *in vitro* models typically neglect host factors. Furthermore, interactions between microbes can be communalistic or mutualistic depending on the context. For example, the same pair of cross-feeding strains can exhibit a range of interactions depending on nutrient availability, from competition in high nutrient conditions to obligate mutualism in low nutrient conditions¹⁶. In general, nutrient addition releases one or both species in a syntrophic interaction from dependence on the other, shifting mutualistic interactions to either commensalistic or parasitic¹⁷. Such parasitic interactions can result in uncontrolled growth of the faster-growing species and exclusion of the slow-grower¹⁷.

By considering these complexities, multiple hypotheses for how cooperation stably exists in the gut microbiome consider the contextual details of cross-feeding interactions¹¹. These explanations converge on two mechanisms that prevent cooperative interactions from forming run-away positive feedback loops: (1) blocking the overgrowth of cooperative species and (2) weakening the strength of cooperative interactions so that coupling between species is dampened. Mechanisms that block overgrowth of cooperative species include the presence of competitive interactions and host-mediated limitations on microbial growth such as immune regulation. Alternatively, the strength of cooperative cross-feeding can be dampened by spatial structure, which increases physical distance between interacting partners and increases the likelihood that sharing occurs between clonemates rather than through cross-feeding. Functional redundancy can also replace a single strong cooperative interaction with several weak ones¹¹. Future studies that begin to account for these unseen factors will help to provide better understanding of the ecology of our microbiome and the role that cross-feeding plays in driving broader community dynamics.

Why and how does cross-feeding occur?

Why and how do microbes release public goods? As to the “why” of it, we must first address whether cross-feeding is an evolved trait or a serendipitous occurrence. In some cases, there is evidence that bacteria are adapted to rely on mutualistic cross-feeding interaction. For example, syntrophic bacteria involved in interspecies H₂ transfer often have highly reduced genomes, lacking key components for anaerobic respiration but specialized towards their given role in the interaction (see below for discussion on H₂ transfer)¹⁸. In another example, a recent paper suggests that certain gut bacteria secrete enzymes for polysaccharide digestion for the benefit of a cross-feeding species, which in turn provides a different benefit to the former organism¹⁹. As described below, the release of public goods can also be serendipitous as a result of cell lysis, overflow metabolism, or other processes.

As to the “how” of public good secretion, there are a handful of proposed mechanisms. High molecular weight polysaccharides and proteins are usually degraded outside of the cell and release sugars available for others. Intracellular metabolites may be released through cell lysis, for example through the action of host or microbial toxins, starvation or lytic phages. Indeed, lytic phage have been shown to play a role in nutrient release in both natural and laboratory settings^{20,21}.

Apart from cell lysis, intracellular metabolites can also be released from live cells. Sometimes this may occur by free diffusion across lipid membranes or ‘accidental’ leakage through transporters. In some cases, overflow metabolism may contribute to the availability of intracellular metabolites to neighbouring bacteria. This phenomenon is based on metabolic models that optimize growth rate, rather than efficiency, under a given nutrient environment²². During overflow metabolism, growth rate under high resource supply is faster using fermentation (thereby secreting acetate) than aerobic respiration²². Overflow metabolism has been largely studied in *E. coli* but is also observed in the gut symbiont *Bacteroides thetaiotaomicron*, which secretes acetate, formate, succinate and propionate as major metabolic by-products²³. These metabolites are all easily derived from fermentative

glycolysis by-products and their accumulation is predicted from metabolic models that allow overflow metabolism.

Another hypothesis for metabolite leakage is that under starvation, cells secrete non-degradable metabolites, such as some amino acids, in order to prevent their toxic buildup²⁴. Alternatively, in a phenomenon called “noise-averaging cooperation”, noisy regulation of metabolic enzymes at the single cell level causes a subset of cells to excrete metabolites that are able to be used by their genetically identical kin²⁵. This sharing of metabolites allows related bacteria to “average out” their communal metabolism and together improve collective growth. However, in a diverse community these metabolites are available to other species and can result in interspecies cross-feeding²⁵. Finally, metabolic flux analysis suggests that secretion of many metabolic by-products is ‘costless’ to the producer under certain conditions, depending on which nutrients are limiting and the carbon-nitrogen ratio in the cell²⁶. In this setting, there would be no selective pressure to prevent release of these metabolites from the cell.

An outline of cross-feeding in the gut microbiome

Metabolites that undergo cross-feeding in the microbiome can broadly be classified into two groups: those used directly in central metabolism (sugars and electron donors/acceptors), and those that are essential nutrients and require biosynthesis or uptake (amino acids, cofactors, vitamins).

In terms of central metabolism, cross-feeding establishes trophic levels (Figure 2a), where primary degraders (first trophic level) with specialized machinery hydrolyze complex polysaccharides, releasing oligo- and monosaccharides accessible to other species. Primary fermenters (second trophic level) liberate these sugars themselves or acquire them from other microbes, and funnel these sugars through glycolysis, producing phosphoenolpyruvate (PEP) that is used for substrate level phosphorylation and generating organic acids (e.g. formate, acetate, succinate) or alcohols (e.g. 1,2-propanediol) (Figure 2b). Next, secondary fermenters (third trophic level) can make use of these by-products in various fermentative or respiratory pathways of their own, generating short chain fatty acids (SCFA: acetate, butyrate, propionate). Finally, molecular hydrogen (H₂) produced by primary/secondary fermenters (Figure 2c) serves as an electron donor for sulfate reducing bacteria (SRB), methanogens and acetogens (fourth trophic level; Figure 2d).

Given the complexity of intestinal biogeography, does the gut microbiome truly function in trophic levels? In support of the notion, some trophic roles are mutually exclusive; for example, no known microbes are both primary degraders and methanogens²⁷. Furthermore, while many bacteria encode the potential to fill multiple trophic roles (e.g., many acetogens also encode extracellular polysaccharide hydrolases), this potential is not realized as bacteria shift to specific trophic roles based on the environment and community context²⁸. Correlational analysis of human microbiome data also identifies trophic networks²⁹, as does *in situ* modelling³⁰. While such modelling does not account for all contributing parameters, a framework dictated by trophic levels allows for coarse-grained understanding

of community function that is too complex to achieve using fine-grained approaches focused on single species³⁰.

First trophic level: Primary degraders sharing sugar in the gut

While there are examples of primary degraders across diverse bacterial phyla in the gut, members of the order Bacteroidales are central to the carbon food web³¹. These bacteria exist at densities ranging 10^9 - 10^{10} CFU/g feces and are comprised of three dominant genera: *Bacteroides*, *Parabacteroides* and *Prevotella*. The primary niche of Bacteroidales in the gut is the degradation of plant polysaccharides that escape digestion by human enzymes in the small intestine. Genes required for growth on a particular polysaccharide are encoded in gene clusters termed polysaccharide utilization loci (PUL), which may include surface proteins that bind polysaccharides (canonically SusD), surface-associated enzymes that cleave them (glycoside hydrolases/polysaccharide lyases, GH/PL, canonically SusG), transporters for import (canonically SusC), and periplasmic GH for degradation into monosaccharides³². Since different PULs are specific to different substrates, each species' capabilities depend on the PULs it encodes. The presence of surface exposed GH, usually encoding N-terminal signal peptidase II sequences for lipoprotein attachment, allows a species to produce public goods³³. These surface exposed GH/PL can also be secreted in outer membrane vesicles based on their lipoprotein export sequence^{33,34}. In this way, outer membrane vesicles can themselves act as public goods.

Due to their role as primary degraders, *Bacteroides spp.* are often considered keystone species in a community with disproportionately large impacts that ripple throughout the ecosystem³⁵. Taking it a step further, PULs themselves are important drivers of community composition. For example, various PUL mutants in *Bacteroides uniformis* support significantly lower growth of cross-feeding firmicute butyrate producers, and thus lower butyrate production³⁶.

It could be tempting to draw a simple model where PUL presence predicts the availability of cross-fed substrates and thus the abundance of secondary fermenters that rely on these substrates. However, empirical data demonstrates that whether cross-feeding occurs depends on the glycan and interacting species involved. There are two factors that contribute to this specificity. The first is the ability of recipient species to import and metabolize the mono- or oligosaccharides derived from the targeted polysaccharide, which requires the necessary machinery to utilize the released sugars. The second factor is how the primary degrader metabolizes the polysaccharide. Different species encoding the same PULs release different types, amounts and proportions of mono/oligosaccharides. For example, while *Bacteroides caccae*, *B. ovatus*, *B. uniformis* and *B. thetaiotaomicron* all metabolize inulin, different amounts of oligosaccharides versus fructose are released³³. Primary degraders can also operate through either selfish or unselfish mechanisms. While unselfish mechanisms produce public goods, selfish metabolism utilizes minimal extracellular hydrolysis to prevent loss to other microbes. Yeast α -mannan degradation by *B. thetaiotaomicron* is one example of the latter, where surface exposed endo- α -mannosidases mediate infrequent cleavage of the mannan backbone, producing relatively large oligosaccharides that are imported for periplasmic endo- and exo- α -1,6-mannanases³⁷. Similarly, *B. ovatus* adopts

a selfish strategy for hemicellulose degradation, but ‘unselfish’ strategies for degradation of other xylooligosaccharides³⁸. Usually, extracellular polysaccharide digestion that provides public goods to other species is thought to be commensalistic or exploitative, i.e. it does not benefit the primary degrader. However, in one specific example a mutualistic interaction has been proposed: extracellular digestion of inulin by GH secreted by *Bacteroides ovatus* is dispensable for its own usage of the fiber yet allows *Bacteroides vulgatus* to feed on the liberated oligo/monosaccharides¹⁹. *B. vulgatus* in turn enhances the growth of *B. ovatus* through an unknown mechanism. The authors propose that this reciprocal benefit provides selection to maintain these extracellular hydrolases in *B. ovatus*.

There is a plethora of other substrates and species involved in cross-feeding polysaccharides in the gut microbiome. One notable interaction contributing to disease is cross-feeding of host-derived sialic acid between *Bacteroides* spp. and enteric pathogens including *E. coli*, *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) and *Clostridium difficile*^{39,40}. *Bacteroides* spp. sialidase activity, which liberates monomers from glycoproteins and oligomers, promotes pathogen expansion in both colitis and post-antibiotic infection models^{39,40}. Other cross-fed substrates include arabinogalactan⁴¹, inulin⁴², pectin⁴³, xanthan gum⁴⁴, and xylitol⁴⁵. Further characterization of the genes and enzymes responsible for metabolizing these complex substrates and the species that utilize the liberated substrates will allow for greater understanding of specificity during polysaccharide cross-feeding.

Second and third trophic levels: Cross-feeding fermentation by-products

After liberation by primary degraders, energy from sugars is harvested through the combined action of members of the second and third trophic levels. Collectively, sugars are funnelled into one of three upper pathways (Entner-Doudoroff; Embden-Meyerhof-Parnas [glycolysis]; Pentose Phosphate) to produce PEP which is directed through various lower fermentative pathways and ultimately generates short chain fatty acids (SCFA: butyrate, propionate, acetate) and gases (CO₂, H₂). Key intermediates are generated from PEP (Figure 2b)^{45,46}. First, PEP can be used to produce fumarate, which acts as a terminal electron acceptor during anaerobic respiration, producing succinate which is subsequently decarboxylated to propionate⁵². Second, PEP can be converted to pyruvate through substrate level phosphorylation, and subsequently fermented to propionate via lactate (acrylate pathway), propionate via succinate, or butyrate via acetyl-CoA⁵¹. Third, acetate is a key intermediate produced via acetyl-CoA hydrolysis or by acetogens using the Wood-Ljungdahl pathway, and used in an alternate pathway for butyrate production using butyryl-CoA:acetate CoA-transferase (acetyl-CoA pathway)⁴⁷. Finally, deoxy-sugar fermentation (i.e. fucose and rhamnose) produces propionate through the propanediol utilization pathway and the key intermediate 1,2-propanediol⁴⁵.

Individual cells do not usually ferment sugar all the way to SCFAs, but instead secrete intermediates as a result of overflow metabolism. Which intermediates are released depends on factors that optimize energy harvest and growth rate, including CO₂/H₂ partial pressures and redox balance (NAD⁺/NADH) in the cell⁴⁶. These intermediates are taken up by other cells and fermentation is completed, producing SCFA. The net

outcome of this cross-feeding is that the three major SCFA (acetate, propionate, butyrate) accumulate to high concentrations in the gut, peaking in the proximal colon, while other fermentation intermediates (e.g. fumarate, lactate, succinate, 1,2-propanediol) do not⁴⁸. Acetate is produced by many bacteria while propionate and butyrate production is less widespread; for example, Lachnospiraceae (e.g. *Anaerostipes*, *Roseburia*, *Eubacterium*), and *Faecalibacterium prausnitzii* are prominent butyrate producers while *Bacteroides* spp., Negativicutes and some *Clostridium* are major propionate producers^{45,47}.

For many species, cross-feeding fermentative intermediates is an integral part of their lifestyle in the gut. Acetate cross-feeding is particularly important, since it is consistently the most abundant SCFA in the gut across populations⁴⁸, and substrate for production of butyrate, a physiologically important SCFA in human health⁴⁹. Underscoring its importance, acetate is also required for growth of most butyrogenic bacteria^{50,51}. Lactate is used to a lesser degree, supporting ~20% of all butyrate production in the gut, in a pathway that first requires oxidation to pyruvate (Figure 2b)^{52,53}. Growing cross-feeding species together often results in higher butyrate levels, growth rate and/or biomass of each species compared to these species cultured alone. For example, *Bifidobacterium* spp. ferment fructooligosaccharides to produce acetate and lactate, which is used by butyrate producing firmicutes including *F. prausnitzii*, *Roseburia*, and *Eubacterium* spp.^{47,54}. Fructooligosaccharide supplementation therefore increases the abundance of both *Bifidobacterium* and butyrate-producing firmicutes^{55,56}. Acetate and formate produced by *Bifidobacterium* spp. are also thought to be important for butyrate producing Clostridia in the infant gut during the transition from breast milk to solid food⁵⁷.

The trophic behaviour of a given species depends on the presence of their cross-feeding partner(s). For example, colonization of a gnotobiotic mouse with *B. thetaiotaomicron* and *Eubacterium rectale* together versus alone results in both species shifting towards a more specialized trophic role; *B. thetaiotaomicron* upregulates a variety of PULs for glycan degradation, while *E. rectale* downregulates GHs and upregulates enzymes for acetate metabolism²⁸. The fitness cost of these pathways is also affected. For instance, genes required for 1,2-propanediol utilization in *Lactobacillus reuteri* confer a fitness burden in the mouse gut that is reversed in the presence of *Bifidobacterium breve*, a 1,2-propanediol producer⁵⁸.

Fourth trophic level: Interspecies H₂ transfer

Molecular hydrogen (H₂) is an important electron sink in many pathways for anaerobic growth. For these bacteria, proton reduction is coupled to ferredoxin, formate, NADH and FADH₂ oxidation, all of which result in H₂ production (Figure 2b-c)⁵⁹. However, the low redox potential of H⁺/H₂ means that these reactions are only favourable in low H₂ partial pressure and its buildup can slow or inhibit growth, usually by inhibiting NADH oxidation (Figure 2c). Removal of H₂ by partner bacteria or archaea, a process called interspecies H₂ transfer, is critical for growth of these bacteria and a classic example of syntrophy. These interactions are often obligate⁵⁹, but facultative relationships also exist if alternative intracellular metabolites can be reduced instead of protons. Nonetheless, use of protons allows higher ATP yield and so is favourable^{59,60}.

Several types of metabolism consume H_2 as part of a strategy to generate ATP (Figure 2d). These include hydrogenotrophic methanogenesis ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$), acetogenesis ($4H_2 + CO_2 \rightarrow CH_3COOH + 2H_2O$; Wood-Ljungdahl pathway), and various forms of hydrogenotrophic respiration⁴⁶. In general, hydrogenotrophic respiration utilizes H_2 as an electron donor for the electron transport chain that ultimately reduces terminal electron acceptors (e.g. fumarate, nitrate, sulfate)⁶¹. A specific type of hydrogenotrophic respiration is dissimilatory sulfate reduction ($4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$), which involves a specific strategy for activating sulfate with ATP and ultimately reducing it to H_2S ⁶². All of these forms of H_2 consumption ultimately generate a H^+ or Na^+ gradient that drives ATP synthase. Hydrogenotrophic respiration cleaves H_2 in the periplasm into protons, which directly contribute to the proton motive force, and electrons, which enter the electron transport chain⁶¹. Additional proton motive force can be generated in some species when oxidation of electron carriers (e.g. ferredoxin) is coupled to translocating H^+ or Na^+ across the membrane by ferredoxin:NAD oxidoreductase (Rnf) or ferredoxin: H^+ oxidoreductase (Ech)^{62,63}. This strategy is used to generate ATP during dissimilatory sulfate reduction and acetogenesis. Alternatively, during hydrogenotrophic methanogenesis, energy conservation occurs during a methyl transfer reaction by the membrane-bound methyltransferase Mtr that translocates Na^+ across the membrane⁶⁴.

These metabolic strategies for H_2 consumption are utilized by diverse and distinct taxa of microbes in the gut. Sulfate reducing bacteria (SRB) predominantly belong to the genus *Desulfovibrio*, while all known methanogens are Archaea and belong to diverse orders including Methanobacteriales and Methanomassiliicoccales, with *Methanobrevibacter smithii* prominent in the human gut⁶⁵. Acetogenesis and other forms of hydrogenotrophic respiration are found in diverse phyla, though acetogens are primarily firmicutes⁶⁰. Importantly, hydrogenotrophic respiration is essential for the virulence of several enteric pathogens including *Helicobacter pylori* and *S. Typhimurium*⁶⁶.

In contrast to SRB and hydrogenotrophic methanogens whose growth is limited by H_2 availability, acetogens utilizing the Wood-Ljungdahl pathway generally have more metabolic flexibility. In addition to H_2 , these microbes can use alternate electron donors for CO_2 reduction including carbon monoxide or organic carbon sources such as sugars or alcohols. Furthermore, many acetogens lack formate dehydrogenase, the enzyme for the first step in the Wood-Ljungdahl pathway (reduction of CO_2 to formate using H_2)⁶⁷. Therefore, these bacteria benefit from cross-feeding on formate produced by other species in the gut⁶⁸. On the other hand, SRB growth is limited by availability of sulfate, which it obtains in part through cross-feeding mediated by *Bacteroides*-encoded sulfatases that liberate sulfate from host glycans (e.g. chondroitin sulfate)⁶⁹.

Based solely on energetic favourability, SRB can outcompete methanogens for H_2 (as long as sulfate is not limiting), and methanogens can outcompete acetogens⁷⁰. Despite this apparent metabolic stratification, acetogens are more prevalent and abundant than SRB or methanogens⁶⁰. The success of acetogens may be explained by their ability to use alternative electron donors to H_2 and other encoded strategies for energy harvest such as polysaccharide degradation. Indeed, in communities that contain methanogens and SRB, acetogens provide only a minor contribution to H_2 consumption⁷¹.

Several examples *in vitro* and in gnotobiotic mice demonstrate that pairing sugar fermenting gut microbes with H₂ consuming species increases cell density and can impact metabolic output of the interacting species⁶⁹. For example, the relative abundances of the methanogenic Archaea *Methanobacteriaceae* and H₂ producing *Christensenellaceae*⁷² are positively associated in different human populations across several studies. *In vitro*, *M. smithii* produces more methane when paired with *Christensenella minuta* compared to other H₂ producers, possibly owing to the high H₂ production of *C. minuta* and physical aggregation with *M. smithii*. In turn, *C. minuta* shifts its metabolism from butyrate to acetate production with potential impacts on host health⁷².

Amino acid cross-feeding

Amino acids are an important currency in microbial communities. They act as an energy source by entering central metabolism, as a nitrogen source for transamination reactions, and as an essential nutrient for amino acid auxotrophs. In contrast to the carbon food web, which centers around Bacteroidales due to their glycan degrading capacity, the nitrogen food web is driven by diverse species including many firmicutes such as *Clostridium*, *Fusobacterium*, *Actinomyces*, *Propionibacterium*, and *Peptostreptococcus*^{31,73}. Indeed, many Clostridia degrade amino acids through oxidative, reductive or coupled pathways, including the Stickland reaction (Figure 3a)⁴⁶. Together, these species drive nitrogen cross-feeding in the gut, which can be exchanged in two forms: ammonium (NH₄⁺) and amino acids.

Ammonium is abundant and microbially produced in the colon, where concentrations increase distally (Figure 3c)⁷⁴. It acts as an important nutrient for *Bacteroides* spp. growth, since some *Bacteroides* spp. cannot grow on amino acids as the sole nitrogen source⁷⁵. *In vivo*, *B. thetaiotaomicron* shows preferential use of NH₄⁺ for arginine and lysine biosynthesis by upregulating alternative enzymes depending on ammonium availability⁷⁵. *Bacteroides* spp. cross-feed NH₄⁺ produced by diverse bacteria during amino acid fermentation (Figure 3a)⁷⁶, or from urea, which is excreted in abundance into the intestine (Figure 3b). In this case, cross-feeding occurs when urease expressing species, including members of all dominant phyla, liberate NH₄⁺ for utilization by urease non-producers⁷⁷.

The second way that nitrogen is exchanged during cross-feeding is in the form of amino acids. More energy can be conserved by using amino acids anabolically, thereby relieving the necessity for amino acid biosynthesis, compared to catabolic pathways that funnel them into central metabolism. Empirical evidence that amino acid biosynthesis is energetically costly leads to the hypothesis that auxotrophs should be common⁷⁸. The loss of amino acid biosynthetic genes is consistent with the Black Queen Hypothesis, which suggests that gene loss provides a selective advantage if the essential function/nutrient is provided in the environment, for example through the release of public goods⁷⁹.

Amino acid auxotrophy is widespread: metabolic pathway analysis suggests that 24%⁸⁰ to 98%⁸¹ of bacteria lack the ability to synthesize at least one amino acid. A more recent analysis of >12,000 communities included in the Earth Microbiome Project, including free-living and host-associated natural communities, identified amino acid auxotrophs in

virtually all communities⁸². Some caution should be taken when interpreting these data because cryptic amino acid biosynthetic pathways can lead to erroneous over-prediction of auxotrophy^{83,84}. Nonetheless, applying the same pipeline to host-associated (e.g. gut microbiome) and free-living species highlights the increased frequency of auxotrophy in host-associated microbes (in Earth Microbiome Project data, 45% vs 25% of species, respectively)⁸². This observation is consistent with increased nutrient availability in host-associated environments.

Despite the increased frequency of predicted auxotrophy in host-associated environments, notable examples of cross-feeding between amino acid auxotrophs are documented in free-living settings including food fermentation⁸⁵⁻⁸⁸. Synthetic communities of amino acid auxotrophs are also used to probe the principles of cross-feeding and mutualism in community ecology. For example, syntrophic growth of 14 auxotrophic *E. coli* mutants demonstrates that sharing energetically expensive amino acids (e.g. methionine, lysine, isoleucine, arginine and aromatics) promotes stronger interactions than cross-feeding inexpensive amino acids⁸⁹. These interactions are dependent on nutrient availability; for example, cross-feeding amino acids between *S. cerevisiae* and lactic acid bacteria is dependent on nitrogen availability to allow *S. cerevisiae* to engage in 'costless' overflow metabolism of amino acids that subsequently become available to auxotrophic Lactobacilli⁸⁷. Cross-feeding experiments using synthetic consortia can also probe how and when cooperation can evolve⁹⁰⁻⁹². Addressing these basic science questions has important implications to understanding resilience to nutrient change and resistance to invasion¹².

In contrast to limited amino acid availability in free-living systems, bacteria in the mammalian gastrointestinal tract may have ample access to protein and amino acids. Thus, is amino acid cross-feeding between auxotrophic microbes relevant in the gut? While there is certainly *in vitro* and *in silico* evidence that gut microbes can cross-feed amino acids^{93,94}, whether this is an important amino acid source *in vivo* is unclear. In the small intestine, dietary protein is abundant and studies using labelled proteins or nitrogen sources indicate that microbes subsist on this source^{95,96}. In the distal colon, fermentable glycans become scarce and amino acids are more frequently utilized for fermentation (Figure 3c). Here, fermentable fiber is depleted, as are SCFA that inhibit proteolytic degradation of proteins⁹⁷, and so amino acid fermentation becomes favourable. It is feasible that the fermentation of amino acids restricts their use by auxotrophs and encourages mutualistic cross-feeding.

There is little direct *in vivo* evidence for amino acid cross-feeding in the gut. One example in a simplified system, the *Drosophila* gut, demonstrates that *L. plantarum* produces lactate through central metabolism, which is used by *A. pomorum* to generate amino acids that are essential for *L. plantarum* growth⁸. While these essential amino acids are usually present in the diet, the authors propose that this form of cross-feeding protects the microbial community from perturbation should the host diet lack these nutrients⁸. Interestingly, the mutualistic interaction also decreases the fly's preference for a protein rich diet through a mechanism mediated by lactate and *A. pomorum*. In the human gut, a similar exchange of lactate and amino acids has been demonstrated *in vitro* between *Lactobacillus rhamnosus* and *Bacteroides caccae*¹³. Finally, in gnotobiotic mice carrying a four membered consortium of engineered amino acid auxotrophs, evidence of cross-feeding was stronger on a low-

protein diet than high-protein diet⁹⁸. This is consistent with the hypothesis that dietary protein can rescue auxotrophic requirements in the gut microbiome.

Taken together, these studies establish the potential for amino acid cross-feeding in the human gut microbiome. Although the relative contribution of these interactions to microbial growth in the human gut requires further study, it is notable that bacterial metabolites of amino acid metabolism have important impacts on host health including obesity⁹⁹, neurologic disorders¹⁰⁰, and drug metabolism¹⁰¹. Future studies that discriminate mechanisms of amino acid cross-feeding *in vivo* will be important to understanding and predicting the contribution of the microbiome to these host phenotypes.

Vitamin cross-feeding

Apart from cross-feeding major carbon and nitrogen sources, micronutrients including vitamins and cofactors are also cross-fed in the gut microbiome. Vitamins are well suited to inter-microbial cross-feeding in the distal gut: (i) they are essential for enzymatic function in vital cellular pathways, (ii) they are energetically expensive to synthesize, so consequently prototrophs regulate biosynthesis and auxotrophs are common, (iii) they are commonly imported from the environment using dedicated transporters, and (iv) in contrast to amino acids and central metabolism substrates, only low concentrations (nanomolar range) are required for growth. As evidence for the latter point, in a genome wide screen of *E. coli* auxotrophs, vitamin auxotrophs emerged as ideal cross-feeding collaborators able to grow rapidly in the presence of a prototrophic partner yet do not grow in their absence¹⁰². The authors attribute this quality to the low concentrations of vitamins required for rapid growth as compared to large amounts of amino acid or nucleotides that are required.

Bacteria synthesize/import and therefore potentially cross-feed multiple B vitamins (thiamine [B₁], riboflavin [B₂], niacin [B₃], pantothenate [B₅], pyridoxine [B₆], biotin [B₇], folate [B₉], corrinoids [B₁₂]), quinones (including menaquinone [K₂], and ubiquinone [CoQ]) cellular antioxidants (glutathione and ergothioneine)^{103,104}. While there is less evidence of cross-feeding antioxidants or quinones, there is extensive literature on cross-feeding B vitamins^{105,106}

Vitamin B₁₂ belongs to a group of closely related compounds known as corrinoids and is perhaps the most widely studied example of vitamin cross-feeding in microbial communities. Corrinoids meet all the characteristics for an ideal cross-fed metabolite: (i) they are important for the growth of many microbes, as ~75% of bacteria encode corrinoid dependent enzymes¹⁰⁷; (ii) roughly half of bacteria are auxotrophs, as corrinoid biosynthesis is expensive, involving ~30 enzymatic steps¹⁰⁸; (iii) import is common, as different forms of corrinoids and their biosynthetic intermediates are efficiently captured by dedicated scavenging/transport systems (BtuBFCD)¹⁰⁹; and (iv) only nanomolar amounts are required for growth. B₁₂ consumed in food is absorbed in the ileum with bioavailability of ~50% depending on the food, and saturated at ~2 µg/meal¹¹⁰. Therefore, depending on diet, corrinoids in the distal colon can be of both dietary and microbial origin. Dietary B₁₂ that reaches the colon is most likely converted into other corrinoids by the microbiome¹¹¹. However, under limiting dietary B₁₂, which can occur in diets low in animal

products, evidence supports that microbial corrinoid production and cross-feeding stabilizes the community. First, direct *in vivo* evidence demonstrates that *B. thetaiotaomicron*, a B₁₂ auxotroph, relies on uptake from the environment, since transporter mutants are outcompeted by the wildtype strain in monocolonized mice but not in mice co-colonized with corrinoid prototrophs; lower-efficiency transporters may substitute when corrinoids are abundant¹¹². Second, a B₁₂ deficient diet has almost no effect on the composition of the gut microbiome, suggesting that prototrophs are able to supply corrinoids to auxotrophs¹¹³, though host storage of B₁₂ in the liver and release through enterohepatic circulation may also contribute⁴⁸. B₁₂ can also be shared with other metabolites involved in central carbon metabolism, such as the exchange of corrinoids and 1,2-propanediol between *Eubacterium hallii* (corrinoid producer and 1,2-propanediol consumer) and *A. mucuniphila* (corrinoid consumer and 1,2-propanediol producer) at the mucosal border¹¹⁴.

Thiamine (B₁) provides a second example of cross-feeding in the gut. Thiamine biosynthesis involves two distinct branches that produce stable intermediates (a 5-membered 4-methyl-5-(2-hydroxyethyl)thiazole ring [THZ] and a 6-membered 4-amino-5-hydroxymethyl-2-methylpyrimidine ring [HMP]) which are coupled together using the enzyme ThiE, then phosphorylated to the active cofactor, thiamine pyrophosphate (TPP). Alternatively, thiamine itself can be phosphorylated to TPP¹¹⁵. Many gut and marine bacteria are auxotrophic for the biosynthesis of both THZ and HMP or for the ability to combine THZ and HMP, while import pathways for these compounds are widely reported¹¹⁶. In a synthetic community, *E. coli* mutants in THZ/HMP biosynthesis or Thiamine-phosphate synthase (ThiE) can support each other by sharing these intermediates¹¹⁷. These observations suggest that thiamine and its intermediates can be readily shared among bacteria.

There is similar evidence of cross-feeding for other B vitamins. *In silico* predictions demonstrate widespread auxotrophy of B vitamins, where ~20% of a typical microbial population (by relative abundance) is auxotrophic for each vitamin, apart from B₁₂ auxotrophy which reaches ~50% prevalence¹⁰⁸. Modelling suggests that B vitamins drive modules of interacting gut microbes based on complementary prototroph/auxotroph networks¹¹⁸. Experimentally, varying dietary B vitamin (B₁, B₂, B₃, B₅, B₆, B₇, B₉, and B₁₂) *in vivo* and *in vitro* supports that prototrophs protect auxotrophs from nutrient deficit^{108,113}. *In vitro*, the concentration of B vitamins (B₂, B₃, and B₅) increases in spent media during growth of human complex communities and adding excess B-vitamins does not increase the abundance of auxotrophs, suggesting that prototrophs can supply auxotrophs under these conditions¹⁰⁸. As an *in vivo* example, in the termite gut, the acetogen *Treponema primitia* requires folate (B₉) for growth, which it receives from other gut microbes including *Lactococcus lactis* and *Serratia grimesii*¹¹⁹. *T. primitia* may in turn produce biotin, pyridoxal phosphate and coenzyme A to support growth of *Treponema azotonutricium*¹²⁰.

Notably, similar to amino acid auxotrophy predictions, prediction of vitamin dependency based on genomic data is not always supported by *in vitro* evidence¹²¹. This may partially be due to the conditional essentiality of vitamins. For example, in many microbes vitamin B₁₂ is essential for growth in minimal medium due to the corrinoid-dependent methionine

Iron, heme and siderophores

Minerals (Zn, Mn, Cu, Se, Fe, S, Co, Cu) are essential for both bacteria and the host for the function of metalloproteins involved in vital processes such as DNA replication, response to oxidative stress and ATP production. These cofactors are derived from the diet, and minerals that escape human absorption reach the colon where they are used by microbes. Bacteria capture minerals such as iron from the environment either directly (*e.g.*, using FeoABC transport systems to transport soluble ferrous Fe^{2+}) or through scavenging molecules (*e.g.*, siderophores or heme/hemophores for insoluble ferric Fe^{3+})¹²⁹. While cross-feeding of iron itself has not been documented in the gut, these scavenging molecules are readily exchanged between species. Further, some siderophores such as yersiniabactin function as metallophores that bind other metals such as Cu, Zn and Mn¹³⁰, expanding the scope of mineral cross-feeding among members of the gut microbiome.

Siderophores are structurally diverse and require a cognate receptor for uptake, including an outer membrane TonB-dependent transporter and periplasmic binding protein/ATP-transporter to facilitate translocation into the cell¹³¹. In the context of the gut microbiome, competition for iron is particularly important as it contributes to virulence of enteric pathogens¹²⁹. During infection, the host secretes iron binding proteins lactoferrin and calprotectin as a nutritional immunity strategy to starve pathogens of iron. Likewise, production of siderophores is not required for colonization of commensals (*e.g.* *Bifidobacterium*, commensal *E. coli*) in a homeostatic state¹³², but is important for competition between commensals and pathogens (*e.g.* *Salmonella*) during infection^{133,134}.

Similar to vitamins, siderophores and heme are energetically expensive to produce and thus some non-siderophore-producing bacteria express receptors that allow piracy of siderophores produced by other species (xenosiderophores). Gut *Bacteroides* spp. encode neither heme nor siderophores and obtain iron through the uptake of heme precursors (*e.g.* protoporphyrin)¹³⁵, transferrin¹³⁶, and xenosiderophores in a species-specific manner¹³¹. During infection or colitis when microbial access to iron is limited, fitness of *Bacteroides* spp. becomes dependent on their ability to capture siderophores from Enterobacteriaceae (*i.e.* salmochelin and enterobactin). Indeed, *B. thetaiotaomicron* mutants defective in the *xusABC* locus responsible for salmochelin/enterobactin uptake exhibit no fitness defect in a healthy gut but are outcompeted during *Salmonella* infection or colitis¹³⁴.

What effect does siderophore piracy have on the fitness of the producing organism? Although a negative impact may be expected, experimental studies of *Salmonella* burden in mice colonized with wildtype or XusABC-deficient *B. thetaiotaomicron* strains reveal no impact on pathogen load¹³⁴. Notably, these studies were performed in conventionally raised mice in which *B. thetaiotaomicron* only represented a small proportion of the microbiome; it is possible that the combined action of many siderophore-utilizing species would impact the fitness of the producing organism. Alternatively, mutualistic cross-feeding can increase the fitness of both parties during siderophore piracy. For example, during polymicrobial intra-abdominal infections, synergism between *E. coli* and *B. fragilis* leads to lesion and abscess formation. This synergism is at least partially attributed to *E. coli* hemoglobin protease (Hbp), which is secreted, degrades hemoglobin, binds heme, and delivers it for

uptake¹³⁷. Hbp can be intercepted by *B. fragilis*, which in turn improves *E. coli* growth through mechanisms that remain ill-defined¹³⁷. Environmental Enteric Dysfunction, a form of undernutrition characterized by overgrowth of proteobacteria, chronic malabsorption, and susceptibility to infection, provides a second example. During Environmental Enteric Dysfunction, mutualistic cross-feeding occurs between *E. coli* and *Bacterioides* spp., where *Bacterioides* spp. enhance carbohydrate access for *E. coli* through the liberation of sialic acid from mucin, and *E. coli* enhances iron acquisition of *Bacterioides* through siderophore secretion, hemoglobin degradation and heme biosynthesis¹³⁸. This interaction is dependent on the combination of malnourished and inflammatory conditions, when *Bacterioides* spp. degrade host mucins and iron is limiting. Understanding the mechanisms and consequences of iron acquisition and cross-feeding during these dysbiotic states could aid in the development of therapeutic strategies that ameliorate microbiome-associated disease risks.

Outlook

Despite the seeming simplicity of microbial cross-feeding, outcomes of cross-feeding in microbial communities are clearly complex. As highlighted in the examples above, these interactions can result in commensalism, exploitation or mutualism, can be context dependent, influenced by the participating species and nutrient conditions, and can introduce a throng of eco-evolutionary conundrums involving community stability and altruism. Depending on context, cross-feeding interactions can be an integral function of a healthy microbiota (for example, through promoting growth of butyrate producers) and also contribute to disease (for example, by promoting pathogen expansion). These disparate outcomes may be inherent to metabolites released as public goods that can be accessed by other members of a microbial community.

Despite these challenges, cross-feeding interactions in the gut are worthy of continued study. Various strategies described here simplify cross-feeding, for example studying pairwise interactions or breaking complex communities into trophic levels. Integrating the findings of these studies create the foundation for developing comprehensive models that integrate cross-feeding into community dynamics predictions. Models incorporating proteomic, metabolomic and genomic data continue to increase in accuracy as they harness advancing technologies such as untargeted metabolomics, stable isotope tracking¹³⁹, and more powerful ecological and computation models (e.g. Ordinary Differential Equation-based models¹⁴⁰, Genome-scale Metabolic Modelling¹⁴¹). However, limitations to these approaches exist, including a lack of integration of host factors such as spatial structure and microbe-host interactions and our limited understanding of the metabolic function of diverse species present in the gut. Integration of these host factors and continued investigation of uncharacterized functions in diverse gut microbes remain important goals. Together, these efforts work towards the goal of understanding the composition and metabolic output of gut microbial communities, the impacts of these communities on their host, and how these outputs and impacts can be manipulated for therapeutic benefit.

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Cross-feeding, the sharing of metabolites between different microbes, plays an important role in shaping the gut microbiome. This review describes ecological and evolutionary implications of cross-feeding and dissects specific mechanisms that enable carbon, nitrogen, vitamin and cofactor cross-feeding.

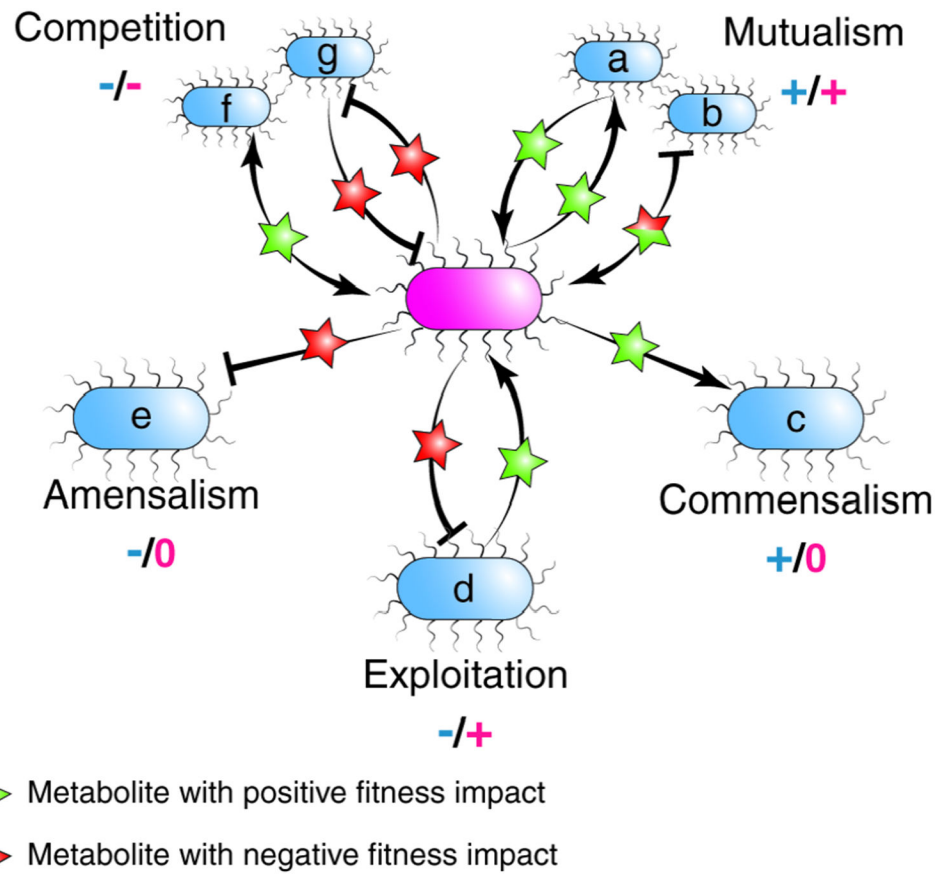


Figure 1. Landscape of microbe-microbe interactions

Microbe-microbe interactions can result in positive (+) or negative (−) impacts on fitness of participating species. These interactions are often mediated by diffusible metabolites, shown here as stars. Cross-feeding can result in mutualism (a-b), commensalism (c) or exploitation (d). These interactions are often through the production of a metabolite by one species that benefits or harms another species (a, c, d, e, f, g), but can also occur when a harmful metabolite to one species is consumed by and benefits another species (b). Amensalism (e) and competition (f-g) can also occur through metabolite exchange, but do not represent cross-feeding interactions.

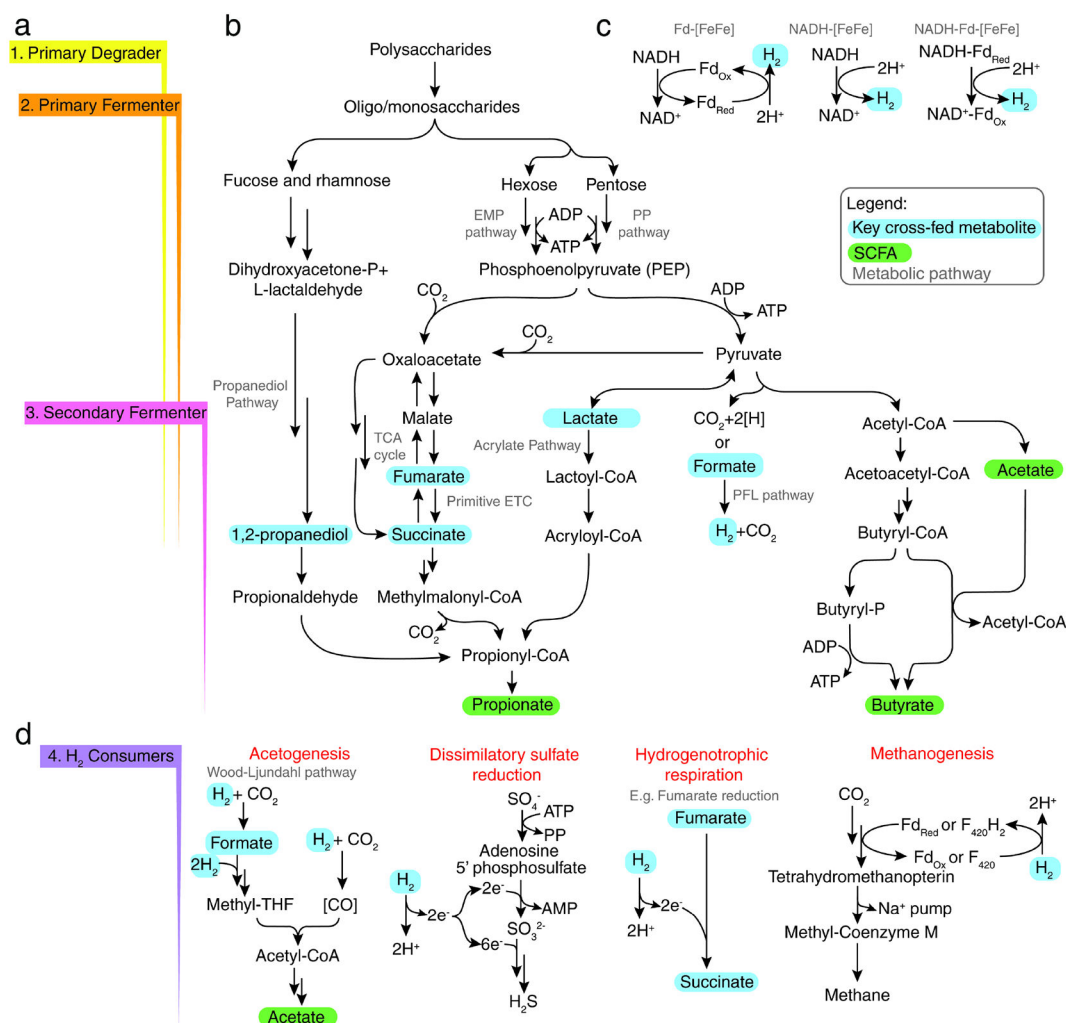


Figure 2. Central metabolism pathways involved in cross-feeding

An overview of central metabolism pathways involved in cross-feeding interactions. (a) Flags along the left show how the pathways in (b) roughly divide into four trophic levels through cross-feeding. Key cross-fed intermediates released by primary degraders/fermenters, highlighted in blue, can be released and utilized by secondary fermenters ultimately for the production of SCFA, highlighted in green. Throughout, H₂ is produced in one of two ways: first, the Pyruvate-Formate Lyase (PFL) pathway that splits formate into H₂ and CO₂, and second through the oxidation of NADH. (c) NADH oxidation can be coupled to ferredoxin reduction and reoxidation by ferredoxin (Fd)-dependent hydrogenase (Fd-[FeFe]), directly by an NADH-dependent hydrogenase (NADH-[FeFe]), or directly by a bifurcating NADH-Fd_{red}-dependent hydrogenase (NADH-Fd_{red}-[FeFe]). (d) H₂ is consumed by various types of metabolism, indicated in red. Pathway stoichiometry is not represented in this figure.

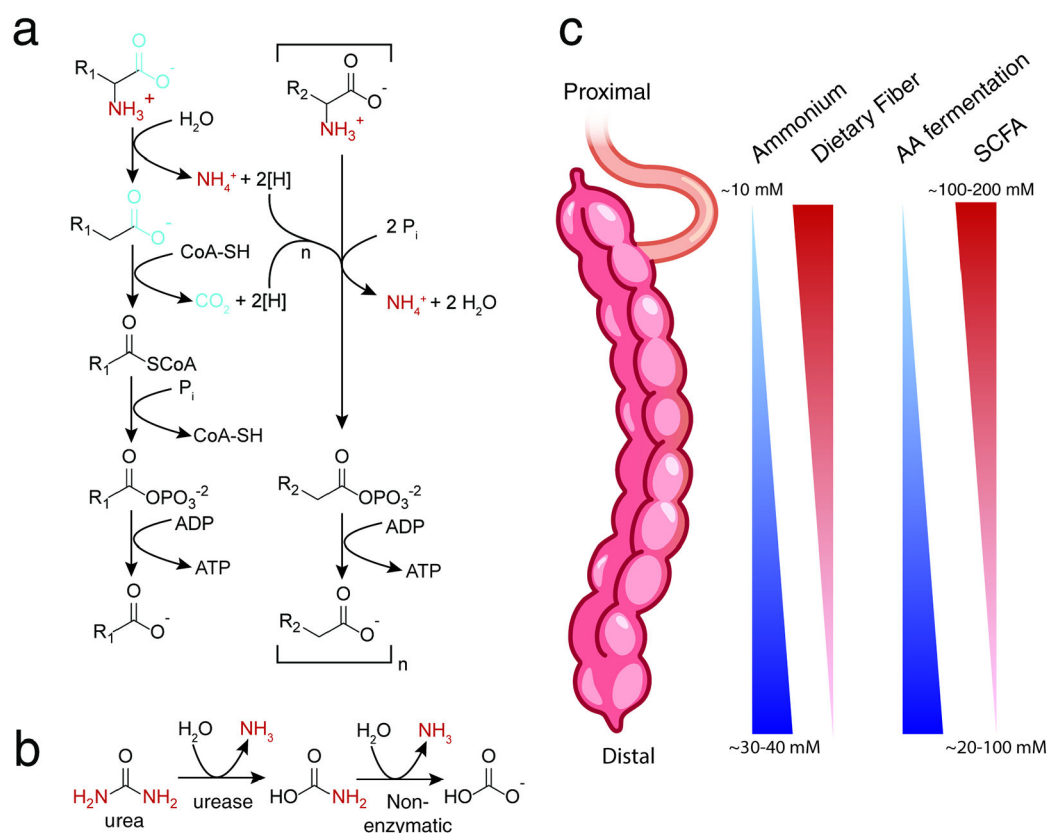


Figure 3. Nitrogen metabolism in the gut

(a) Stickland metabolism couples reduction of one amino acid (left) with oxidation of a second amino acid (right) via electron carriers indicated by [H]. Deamination of the amino acids results in production of carboxylic acid intermediates and ammonium. Stoichiometric equivalents are indicated by n . (b) Deamination of urea by ureases also produces ammonia. (c) Nitrogen and fiber metabolism vary along the length of the colon. Whereas ample dietary fiber in the proximal colon leads to high concentrations of SCFA, amino acid fermentation is more common in the distal colon. Ammonium and SCFA concentrations shown are estimated from human samples⁷⁴.