Review

The Road Not Taken: The Rumen Microbiome, Functional Groups, and Community States

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The rumen ecosystem represents a classic example of host–microbiome symbiosis. In this obligatory relationship, the host feeds on plant fibers that can only be degraded through a set of complex metabolic cascades, exclusively encoded in rumen-associated microbes. These various metabolic pathways are distributed across a multitude of microbial populations. Application of basic ecological principles to this ecosystem can contribute to profound understanding of the rules that shape it. Here, we discuss recent studies by examining the mapping between host attributes, rumen ecosystem composition, and functionality to propose simple, yet powerful concepts to guide the interpretation of microbiome data and enable a better understanding of how the system responds to perturbations.

The Functional Groups Concept and the Rumen Ecosystem

The metabolism associated with rumen ecosystem functions is generally consistent [1–3]; nevertheless, species and taxonomic identity may vary between studies. The reasons for these inconsistencies could stem from both biological and/or technical reasons (Box 1). Hence taxonomy alone cannot be used to define and interpret the state of a given microbiome and its connection to the attributes of the host. Consequently, this misconception begs a conceptual change that should avoid taxonomic interpretation associated with these community states while focusing only on the functions that define them [4].

In this communication, we propose to use **functional group concept** [5] (see Glossary) to complement and bypass the barriers encountered with the taxonomic approach (Box 1). The idea to approach community ecology with the actual traits of organisms and not their taxonomy has been raised before to better interpret and identify general roles that govern community structure [5,6]. In the context of microbiomes, our proposed concept considers microbial functional groups as biologically meaningful clusters of bacterial diversity that could encompass multiple microbial 'species' sharing a specific metabolic function. Functional groups can be defined in terms of the metabolic inputs, that is, electron donors and biomass-building blocks, and metabolic outputs, that is, end-products for the entire rumen metabolic cascades thereby driving energy flow through this ecosystem [6]. One functional group can therefore be defined as the organisms that, together, carry out the succinate pathway, which takes pyruvate as an input and reduces it to succinate as intermediate and propionate as the output [7]. Another example is the acrylate functional group, which takes lactate as an input and produces propionate as output [8]. Significantly, there is no direct mapping between these functional groups and the phylogeny of the microbial species since the same metabolic pathways can be performed by microbes belonging to distant phylogenetic lineages. For example, phylogenetic

Highlights

Rumen microbiomes exhibit repetitive patterns that are connected to their host attributes and can be regarded as community states.

The functional groups concept serves to analyze ecosystem function and community states, as different microbial lineages can share functions; thus, combining taxonomy into functional groups enables better interpretation and understanding of the rumen ecosystem.

Key junctions of functional groups at the final steps of electron flow of the rumen ecosystem can explain observed alternative community states.

Niche modification by functional groups shifts between rumen microbiome community states.

Host genetics and diet are connected to rumen microbiome composition and can influence rumen microbiome community states.

Feedback loops of specific functional groups during rumen microbiome succession can determine rumen community states.

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Box 1. The Gap between Phylogeny and Functional Annotation

We recognize many inconsistencies between microbial taxonomy and functional annotations. This partly stems from the fact that the species concept is vague in the microbial world and the inconsistencies are amplified by the way we analyze microbial communities. For example, two different strains of the same microbial species could have different function-alities that would be shared by more distinct lineages [61,62]. Moreover, our methods for assigning taxonomies when studying complex microbial communities serve to amplify these inherent problems. Most research of microbial communities uses the ribosomal 16S rRNA gene to define taxonomy. Traditionally the different 16S rRNA reads are clustered at 97% similarity to define operational taxonomic units (OTUs) at the species level [63]. In evolutionary terms, this distance allows high levels of clustering between very divergent organisms. Moreover, microbiome studies using 16S rRNA taxonomic annotation reveal discrepancies between this annotation and the gene content of the microbiome [64]. Such redundancies and discrepancies between how taxonomic annotation translates to metabolic functions are also apparent in rumen microbiome studies. Taxis *et al*. highlighted these by overlapping enzymatic databases to both rumen metagenomics data and taxonomic annotation and noted similarities at the metabolic level between taxonomically different microbial communities [65].

It should be noted that our current knowledge of microbial functions is limited both by the narrowed fraction of cultivable microbial species and the current depth limitations of metagenomic sequence annotations [66]. Indeed, although metagenomics annotations are constantly improving, and uncultivable microorganism are being isolated and fully sequenced, we still do not grasp most of the metabolic richness existing in metagenomes. This, in turn, might shadow the simplicity of functional groups by introducing higher metabolic complexity into this concept as well as to the functionality of community states.

distances of different orders are involved in the methanogenesis process. Methanogenesis is a fundamental rumen metabolic process situated at the end of rumen electron flow and carried out by a considerably cohesive taxonomic group belonging to the same archaeal phylum of Euryarchaeota. Indeed, while methane is the only single output metabolite, the input metabolites for its formation vary, creating at least three different functional groups scattered across large phylogenetic distances of different orders [9]. These include: (i) hydrogenotrophic methanogenesis, where H_2 and CO_2 are the input metabolites [10], (ii) methylotrophic methanogenesis, where the input metabolite is a methylated compound [11], and (iii) acetoclastic methanogenesis, where the input metabolite is acetate [12,13]. The breadth of functional groups reaches well beyond the order level and encompasses more distinct phylogenetic groups. For example, the acrylate pathway, mentioned above, exists in two rumen-inhabiting bacteria, *Megasphaera elsdenii* [14] and *Coprococcus catus* [8], the taxonomic classes of which are separated by hundreds of millions of years of evolution (Negativicutes and Clostridia respectively) [8]. Nevertheless, these bacteria from two distinct taxonomic classes can inhabit the same ecological niche, as they were reported to increase in abundance under the same habitat conditions [15]. Similarly, the succinate pathway is carried out by taxonomically distinct microorganisms at the phylum level, that is, in the phylum Firmicutes, *Selenomonas ruminantium* (class Negativicutes) [16] and *Succiniclasticum ruminis* (class Clostridia) [8,17], and in the phylum Bacteroidetes [8]. Similar functions were also found at phylogenetic distances as high as domains, such as the acetogenic functional group (utilizing H_2 and CO_2 to acetate) which is scattered across the bacterial domain.

Although microbial specification exists in the rumen ecosystem, the above examples essentially highlight the functional redundancy, scattered across vast phylogenetic space (Figure 1). This redundancy could lead to different microbial compositions but with the same ecosystem functionality, thus rendering conclusions and comparison between different ecosystems and studies extremely challenging. As an alternative approach, we propose to employ functional groups as a means to describe and comprehend the rumen ecosystem and potentially any other microbial ecosystem. By using the functional groups concept, one gains the ability to describe ecosystem functionality and composition at the same time. Furthermore, by using this concept, both the ecological niche that is inhabited as well as the function carried out by the

Glossary

Community states: assemblages of different functional groups. Microbial community state displays resistance to changes due to ecological feedbacks, unless perturbations are strong enough.

Deterministic processes in community assembly: deterministic processes will always result in the same community composition given the same initial conditions. One example of a deterministic process is the sharp change from aerobic to anaerobic environment in the rumen during the first days of life that is followed by a change in species composition from facultative anaerobes to anaerobes.

Electron sinks: represent the final destination of the electron flow in an energetically favorable reaction (from electron sources).

Food web: the interconnection of food chains: the initial materials and their secondary products that can be consumed or produced by multiple organisms in a defined ecological environment.

Functional group: cluster of microorganisms that are highly similar or identical in the assortment of their input and output metabolites.

Functional group concept: an approach to study microbial ecosystems in which each microorganism present in the environment is sorted into functional groups as a function of their metabolic inputs and outputs.

Historical contingency: a series of internal and external timeline events leading to various outcomes of different microbial community states and ecosystem functions.

Niche modification: alteration of niche dimensions as a result of microbial species growth and persistence.

Niche: the overall habitat requirements that allow a microbial species to grow (reproduce) and to persist. The environmental conditions and resources that define the requirements for microbial species to persist are termed niche dimensions. The fundamental niche is defined by the full range of niche dimensions, and the realized niche comprises the actual dimensions that a microbial species utilizes as a result of
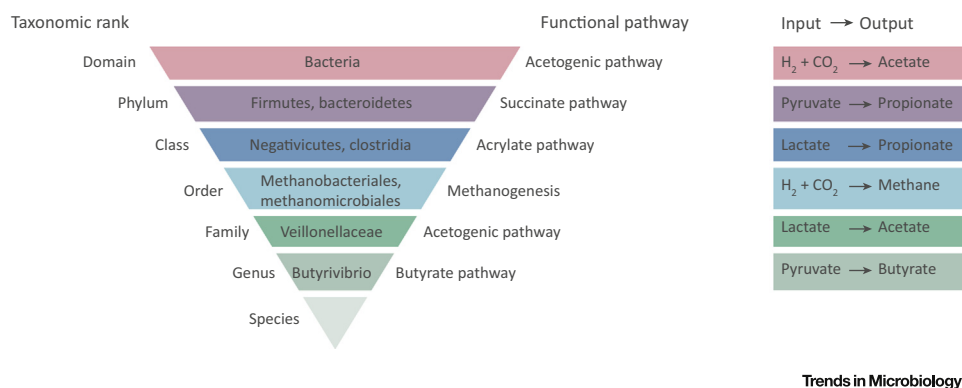
Figure 1. Examples of Different Taxonomy Ranks Regrouping Microorganisms Performing the Same Function. This illustrates the fact that metabolic pathways are spread across numerous phylogenies [18] and emphasizes how misleading taxonomic assignations could be with regard to functionality. The examples given here do not exclude the possibility of other microorganisms from additional phylogenies that could perform the same function. The acetogenic pathway utilizes H_2 and CO_2 to form acetate via acetyl-CoA. The succinate pathway takes pyruvate as an input and reduces it to succinate as intermediate to propionate. The acrylate pathway transfers electrons from lactate to propionate. Methanogenesis regroups three pathways that all produce methane using different input metabolites; the hydrogenotrophic pathway is represented here. The acetogenic pathway performed by the Veillonellaceae family turns lactate into acetate [18]. The butyrate pathway performed by the *Butyrivibrio* genus turns pyruvate into butyrate via acetyl-CoA [19].

resident organisms are described. These examples show that rumen microbes, when divided into functional groups, are distributed across metabolic systems akin to **food webs**, where functional groups are interconnected by the flow of the biomass building blocks together with electron donors and acceptors.

Bifurcating Connections and Niche-modification Could Lead to Different Community Compositions

A crucial feature of the functional groups constructed across rumen food webs is the fact that they can have bifurcating connections or junctions. Ecoevolutionary models argue that competition is the central force shaping microbial community structures [20,21]. Indeed, these junctions emerge when multiple pathways compete for the same substrate, leading to the possible competitive exclusion between functional groups. A fundamental property of microbiomes is that the input and output metabolites that define the functional groups are dynamic and are constructed by the activity of the microbes. The availability of these input and output metabolites can create ecological **niche modification** that can push communities from one composition to the other.

A straightforward example of this concept was reported in a study by Jami *et al*., which characterized the rumen microbiome from birth to adulthood [22]. In this study, extreme compositional changes were found in the rumen microbiomes of 1- and 3-day-old animals that were fed the same diet and exhibited near-identical physical characteristics. These two community compositions varied significantly over a course of 24 h: there was a change from a high prevalence of aerobic and facultative anaerobic bacterial taxa in the 1-day-old calves to strict anaerobes in the 3-day-old calves. These findings suggested that the aerobic bacteria used all the available oxygen in the rumen during the first 2 days of life, thereby modifying the niche to be anaerobic. This niche modification further enabled the expansion of anaerobic microbes at the expense of the first aerobic colonizers. Hence, by modifying their niche, the

interactions and pressures from other species.

Portfolio effects: increase in biodiversity of the ecosystem creates a functional buffering and accounts for its stability while facing perturbations due to the averaging and compensation of fluctuations in species' properties.

Priority effects/founder effects: the order of colonization of the ecosystem influences the outcome of its structure and function.

Stochastic processes in community assembly: these processes depend on a collection of random variables and therefore have the potential for various outcomes in community taxonomic composition.

aerobic microbes changed the community composition. Another example of niche modification driving community composition was also recently shown to drive diurnal oscillation of the rumen microbiome. In this study [23], marked changes in taxonomic composition and functionality of the rumen microbiome were observed as a function of time after feeding. The authors suggested that these are community assembly cycles that are driven by niche modification. By cross-incubating metabolites and organisms from different diurnal time points, the authors showed that the metabolites released by microbes at different hours of the day are sufficient to reproduce changes in community function similar to those observed *in vivo* [23].

Hence, the input and output metabolites of various rumen metabolic groups can drive marked changes in community composition. In this regard, the functional groups that are situated at the end of the electron chain serve to drain most of the rumen metabolism that is carried out by functional groups at the higher energetic levels, which renders them highly important energetic junctions that could influence the ecosystem as a whole (Box 2 and Figure 2). Hydrogen, produced during microbial fermentation [24], is a major junction connecting different functional groups in the rumen ecosystem where changes in hydrogen partial pressure, via niche modification, divert the abundance of one functional group over that of the other (Figure 3).

A good example of such changes is seen in the hydrogen-utilizing functional groups. As mentioned above, hydrogen is utilized in the rumen by several functional groups: (i) by the group using hydrogenotrophic methanogenesis for the reduction of CO_2 to methane, (ii) by the group using the succinate pathway for the production of propionate, or (iii) by the acetogenic functional groups that use hydrogen for the reduction of CO_2 to acetate. A recent study

### Box 2. Food Webs of the Rumen Ecosystem

In the rumen, metabolic cascades are carried out by the microbial community in a complex and coordinated manner, whereby successive food webs of cross feeding exist between different rumen microorganisms. These cascades supply basic metabolites to the hosting animal. Indeed, microbial production of both short- and long-chain fatty acids serves up to 70% of the animal's energy needs [2,67,68].

Almost all of the cow's ingested feed is plant material, which is composed of various polysaccharides that are degraded and fermented by the rumen microbial community. These include cellulose and hemicellulose, pectin, and starch. The primary polysaccharide degraders are bacteria, mainly from the genera *Ruminococcus*, *Fibrobacter*, *Butyrivibrio*, *Ruminobacter*, *Lachnospira*, and *Prevotella,* but saccharolytic microbes are also present from the domains of protozoa and fungi [2,3]. The sugar monomers, the products of their combined enzymatic degradation, are then either fermented directly to volatile fatty acids or, via intermediate molecules (such as succinate, lactate, and fumarate), by key microorganisms belonging to the genera *Selenomonas, Veillonella, Coprococcus*, and *Megasphaera* [3,18].

Proteins and lipids, originating either from the plant material or from the microbes themselves, are also fermented and degraded in the rumen. Proteolytic activity is performed by a vast range of bacteria, such as *Prevotella*, *Butyrivibrio*, *Streptococcus*, *Bacteroides*, *Selenomonas*, and *Ruminobacter,* but also by protozoa [2,69,70]. Lipolytic bacteria, such as *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica,* and protozoa hydrolyze the lipids into smaller molecules, for example glycerol, which will be converted to propionate and saturated fatty acids via possible microbial biohydrogenation [71,72].

The hydrolysis of macromolecules and microbial fermentation produces a high amount of hydrogen, which, if accumulated in an unregulated manner, would inhibit microbial metabolism and block the metabolic cascade [ii] [2,3]. Utilization of hydrogen is performed mainly by methanogens (archaea) and by acetogens, as well as by sulfate- and nitrate-reducing microorganisms to a lesser extent [28,73,74], and results in the production and emission of various gases by the animal, that is, methane, hydrogen sulfide, and ammonia. These microorganisms are essential in generating **electron sinks** and represent an essential driving force for the entire food chain.

This food-web hierarchy of consumption, from the macromolecules down to the final reducers, also gives rise to significant feedback interactions between the primary degraders and the reducers [75,76].
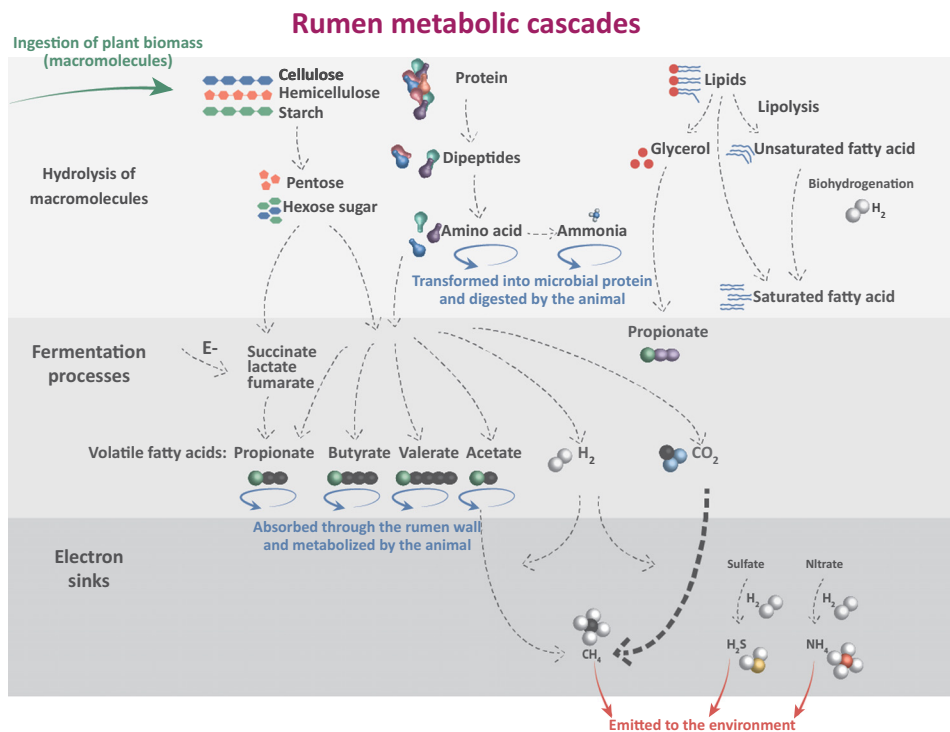
Figure 2. Major Metabolic Cascades of the Rumen Microbiome. Energy harvest from macromolecules undergoes three main stages in which metabolites are transferred among microbes. Green arrows depict ingested biomass, blue circles indicate absorption by the animal, and red arrows represent emissions into the macro-environment. Broken arrows show the cascade of energy flow across the entire metabolic cascade.

demonstrated that the amount of hydrogen produced *ex vivo* by the rumen microbiome immediately after birth is much higher than that produced by the rumen microbiome of adult animals [25]. This is accompanied by the presence of acetogenic functional groups only during the first days of the animals' life, which decrease to very low abundance in the rumen of adult animals [26]. Acetogenic bacteria use the reductive acetyl-CoA or Wood–Ljungdahl pathway to reduce carbon dioxide, under high hydrogen pressure, to acetate [27,28]. Upon reduction of the hydrogen pressure subsequent to its utilization by several functional groups early in life, the acetogenic functional group becomes thermodynamically unfavorable in a new low-hydrogen-modified habitat and is outcompeted by functional groups such as the hydrogenotrophic methanogens, which are not dependent on a high pressure of hydrogen [29]. Therefore, the conventional and most plausible explanation for this phenomenon is exclusion of this functional group due to niche modification of hydrogen pressure [30]. Moreover, competition for hydrogen and niche modification of hydrogen partial pressure also exists within the rumen methane-producing functional groups, since hydrogenotrophic methanogens from the order Methanosarcinales are thought to be more sensitive to low hydrogen pressure when compared to methanogens from other orders, due to the existence of cytochromes in their methanogenesis pathway [29]. Indeed, a recent study documented that species of the order Methanosarcinales are present in the early life of the host, when hydrogen pressure produced by the *ex vivo* rumen microbiome is high, but that they decrease tremendously in abundance in the rumen of 2-week-old animals, when hydrogen pressure produced in the microcosms was lower [25]. Another example of competition for reductive power that results in changes in hydrogen-utilizing functional groups is evident in methane- and propionate-producing functional groups. When
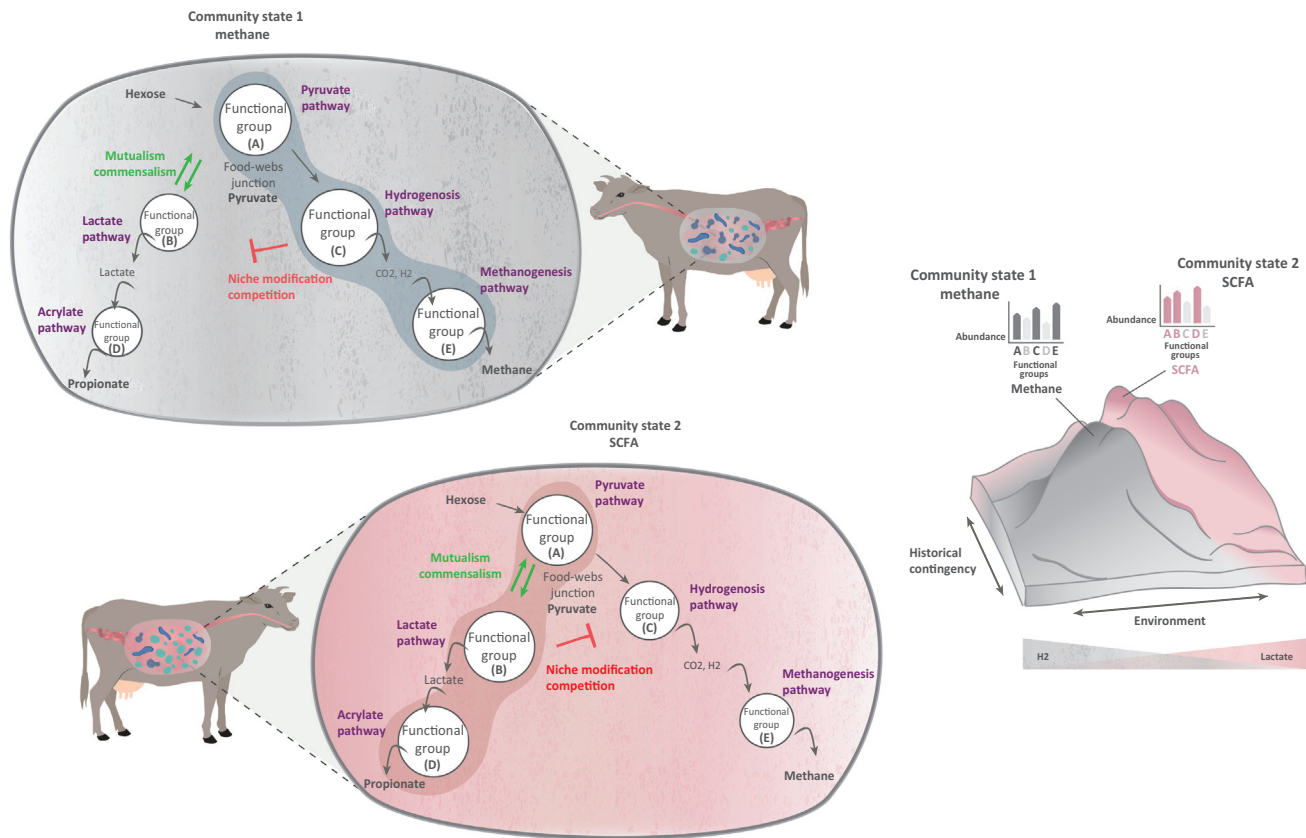
Figure 3. The Emergence of Alternative Stable Community States in the Cow Rumen. *Left panel*. The wording in upper-case letters refers to a schematic illustration of two community states that are composed of a combination of functional groups and metabolic cascades, leading to different ecosystem functions. The top illustration, in gray, shows an alternative stable community state that is governed by functional groups A, C, and E; it leads to a higher production of methane than occurs in the bottom illustration, in pink, where an alternative stable community state is governed by functional groups A, B, and D, leading to a higher production of short-chain fatty acids (SCFAs) (butyrate and propionate). Potential microbial interactions that can influence the emergence of community states could be negative (in red) or positive (in light green). Actual functional groups and metabolites, of which these community states are composed, are represented in dark green, as reported by Ben Shabat *et al*. and Kamke *et al*. [15,49]. These alternative community states are composed of either lactate-producing and -utilizing functional groups (lower pink illustration) that result in a higher production of SCFAs (butyrate and propionate) or hydrogen-producing and -utilizing functional groups (upper gray illustration) that result in a higher production of methane. *Right panel*. Three-dimensional illustration of alternative community states that are composed of different abundance combinations of the functional groups A, B, C, D, and E. Pink represents the community state composed of lactate-producing and -utilizing functional groups with ecosystem function producing higher amounts of SCFAs. Gray represents the community state that is composed of hydrogen-producing and -utilizing functional groups that produce a higher concentration of methane. The arrows represent the potential forces that can influence the emergence and maintenance of these community states such as environmental factors (including host genetics) and historical contingency effects.

hydrogenotrophic methanogenic functional groups are inhibited by antibiotic supplementation in the rumen, an increase in propionate production is usually observed. This phenomenon can be explained by the combined release of propionate-producing functional groups from competition for electrons that are used for fermentation and increase in hydrogen pressure, due to the exclusion of the methanogenesis competitor [6,26,31,32]. Hence, it appears that the hydrogen junction – located at the final steps of the electron flow of the rumen food webs – is central for energy flow through the system, ecology of the microbiome, and consequently interaction with its host.

It should be noted that, despite the need to simplify, functional groups are not only defined by electron flow of rumen thermodynamics but are connected to the ecology of the organisms that

are composing them. This connection implies that the prosperity of functional groups is also linked to the ecological relevance of its host organism to a specific habitat. For example, the presence of hydrogenotrophic methanogens is not only linked to hydrogen pressure but also to the pH that could inhibit their activity [33]. Hence, these two forces, thermodynamics and ecology, interact to determine the existence and abundance of each functional group as the building blocks of the rumen food webs. The meaning of these thermodynamic ecological junctions is that alternative trajectories could be taken by functional groups across the food webs, by modifying the ecological niche via resource utilization or metabolite secretion. These, in turn, can create different community compositions of the rumen microbiome, thereby resulting in distinctive functional landscapes (Figure 3 and Box 3).

Rumen Microbiome Community States That Are Connected to Host Attributes Could Result from Competition between Functional Groups

The theory of alternative stable community states [34] infers that microbiomes can exist under multiple **community states**. These alternative community states, when established, are stable and could be resilient to perturbations but could also transit from one alternative state to another under strong perturbation shifts via possible alternative transient states [35–37]. Such microbiome community states may also have different functionalities and therefore could also be connected to many host attributes. Several studies have addressed the connection between composition of the rumen microbiome and host attributes, such as the ability to harvest energy from feed and its potential methane emission [15,38–45]. Some of these studies specifically pointed to compositional changes in the last steps of the electron flow of the rumen ecosystem, for example, in the methanogenic community [15,42].

In this context, Ben Shabat et al. [15] described rumen microbiome states that are connected to the ability of the host to harvest energy from its feed, in addition to many other attributes. In their experimental setup, the authors ensured the homogenization of many variables, such as diet, which is known to dramatically affect microbiome structure [46–48]. Despite the stringent homogenization of parameters, significant and consistent differences could be detected in microbiome structure and functionality. These differences were mainly situated at the final steps of the electron flow through the rumen ecosystems, namely, the electron transfer from hydrogen, lactate, and succinate to methane and end-fermentation products, such as acetate, butyrate, and propionate, thus pinpointing the functional groups at the end of the electron chain as the main drivers (Figure 2 and Box 2). Specifically, in the rumen microbiomes of energetically efficient animals compared to inefficient animals, short-chain fatty acids (SCFAs) such as butyrate and propionate were found in higher concentrations, whereas methane concentrations were lower. These findings were compatible with the enrichment of the acrylate functional group that transfers electrons from lactate to propionate, together with enrichment of specific species that code for it, such as M. elsdenii and C. catus in efficient microbiomes. In inefficient microbiomes, hydrogen-utilizing functional groups such as hydrogenotrophic methanogenesis and the succinate pathway, were enriched. These observations of different ecosystem functions are in agreement with the potential differences in energetic balance of the hosts, since SCFAs are valuable for the energy needs of the host (enriched in efficient animals), while methane is emitted into the atmosphere together with its retained energy (enriched in inefficient animals) [1,6,26]. These two findings suggested that a bifurcating junction for electron flow located between lactate and hydrogen-producing fermentation pathways defines these microbiome compositional states (Figure 3).

The consistency of these rumen compositional and functional differences over multiple individuals suggests that they are stable and thus can be viewed as alternative community states. Alternative community states, emerging from the electron flow junction located between lactate and hydrogen-producing fermentation pathways and giving rise to alternative animal host

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Box 3. What Factors Can 'Tip the Scales' in Favor of One or Other Community State?

Early-life events during rumen microbiome development could also shape the community states in the adult rumen community [55]. Indeed, a recent study demonstrated an effect of early-life dietary intervention on microbiome development in lambs [77]. From the ecological perspective, these events could potentially be fundamental in defining rumen community states under the niche modification concept. It has long been recognized that so-called '**priority effects**' can lead to alternative community stable states [78] in microbial communities. Priority effects have been documented in plant communities, in which the order of arrival of early species influences the set of species that will dominate future stages of the assembly process [79]. This concept was recently examined in a controlled manner in mice where colonization order of species affected the outcome of community assembly [80].

This means that **historical contingency** during colonization (an early colonization event) can propagate towards the climax community. In the context of rumen microbiomes, it is well established that rumen development occurs in a **deterministic** manner, meaning that it follows the same general patterns across different individuals and that the rumen ecosystem converges to exhibit the same general composition as the animals mature [22,55,81]. Nevertheless, although the rumen microbiome is more similar among adult animals, there is still a large variety between individual animals, even if they experience the same husbandry regime [22,82]. Historical contingency or founder effects can lead to long-term consequences for the rumen microbiome, when feedbacks exist that maintain and reinforce the dominant functional groups while holding competing ones at low frequency. Feedback loops, generated by specific functional groups, could arise at early stages in the developing rumen and intensify with time/age. In this context, several studies detected organisms in the undeveloped rumen just after birth, belonging to functional groups that form the foundations of the adult rumen [22,55,83–86]. This suggests that most alternative trophic chains exist in early life just after birth, and environmental triggers may enrich for specific groups, thereby determining the phenotype of the adult rumen microbiome. Environmental factors such as diet and host genetics could represent triggers enriching for specific functional groups. Dietary composition was connected in various studies to rumen microbiome composition [47,48,87,88]. A recent report, using a longitudinal study design that alternated between two different diets, has shown that dietary alternation mainly affects the most abundant microbial taxa, potentially by changing the composition of available resources as well as environmental factors such as redox potential [89]. Host genetics was shown to be associated with rumen microbiome composition in several studies that examined rumen microbiome variation in different breeds types [40,90,91]. More recently rumen-specific taxa were found to be heritable and associated with host genetics in the same breed and dietary regime [92]. Hence, diet and host genetics, together with other variables such as dispersal limitation, could act as determinants that influence the succession and development of the rumen microbiome.

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attributes, were also reported in studies that examined methane yield in sheep [49,50]. The authors of these studies described similar findings: namely, different community compositions are associated with metabolic junctions at the end of the trophic chain that are connected to ecosystem function. The same functional groups were determined to be associated with these community states where lactate-producing functional groups represented by *Sharpea* spp. and lactate-utilizing functional groups represented by *Megasphaera* spp. were highlighted as important drivers of electron flow towards a reduction in methane production and an increase in SCFAs such as butyrate. Therefore, bifurcating connections of functional groups centered around lactate, and hydrogen could be identified as a central food-web junction [49]. Overall, these studies together indicate that junctions of electron flow at the end of the trophic chain (hydrogen-utilizing, lactate-utilizing, and methanogenesis [51]) lead to possible competitive exclusion between two community states that are interconnected by functional groups and affect host attributes (Figure 3). Different factors such as environment, host genetics, or more stochastic forces such as historical contingency could influence the dominance of one alternative state over the other and are discussed in Box 3. It should be noted that, in different community states, there is an enrichment of one functional group over the other and not necessarily complete exclusion of functional groups[i] [16,24]. Hence, these alternative trophic chains coexist within the rumen ecosystem but the balance among them shifts in each state. Consequently, their output products are the average result of multiple parallel trophic chains assembled in each of these structured environments. The existence of these coexisting alternative trophic chains could have significant impacts on ecosystem function, not only because they could differ in their metabolic efficiency, but also because they could potentially

serve to buffer the system against changing environmental conditions (e.g., changes in diet) by means of **portfolio effects** [52,53].

Although these patterns of community states have been reported across several studies, their resilience and stability to perturbations should be further studied *in vivo* and *in vitro* over extended periods of time in order to further strengthen this concept. In this regard, recent attempts to modulate host energetic efficiency by cross transfusion of rumen microbiomes between efficient and inefficient animals suggest that microbiome composition has high re-establishment capacity and resilience to perturbation. In one study, after replacement of 95% of the rumen content in four animals, only a transient effect was observed on microbiome composition and host phenotype, and a final return to microbiome state and host phenotype similar to the pre-exchange ones was observed after a period of 10 days [54].

These findings suggest that the composition of the rumen microbiome is highly resilient to perturbation and that even a small fraction of the initial microbial community in the rumen has the potential to re-establish itself. One plausible mechanism for such resilience could involve the **founder effect** via niche modification. In this scenario, the fraction of the remaining rumen community would create environmental conditions that cement its own re-establishment and prevent invasion of a secondary community. Hence, when a more thorough and comprehensive purging of the initial community occurs, one would expect other forces to govern the establishment of the colonizing transfused community. These forces could be the **stochastic** initial abundance of each community member as well as potential environmental effects such as host genetics and the immune system [55] that will affect each initial transfused community in a different manner. In this latter scenario, one would expect high variability of the established community after transfusion. Such a pattern was recently reported: after a period of 28 days post-transfusion, high individual variability of microbiome composition was observed. In this study, a rumen transfusion was applied to 16 animals that underwent intense washing of the rumen environment before transfusion to assure effective removal of the rumen content [56]. These studies suggest that the establishment and stabilization of rumen microbiome community states could be the result of microbiome niche modification mechanisms driven by functional groups and by hydrogen and lactate concentrations.

Concluding Remarks and Future Perspectives

Community states of the rumen microbiome are linked to their host attributes; for example, a community state can allow more chemical energy to be channeled into the hosting animal and can be connected to higher feed efficiency versus an alternative community state that will contribute to more methane emission to the atmosphere and will be connected to lower feed efficiency. The literature reviewed here suggests that competition between the functional groups that are situated at bifurcating junctions of the rumen food webs located at the end of the electron chain are most prone to contribute to balancing both structural and functional outcomes of the microbial community. We pointed at microbial interactions as major contributors to the establishment of rumen community states and highlighted niche modification as one of the main mechanisms for this establishment. These, together with host genetics and environmental factors affect the cascades of rumen microbiome succession and the emergence of different community states.

Identification of alternative community states and the forces that determine their assembly could help us for designing rational intervention strategies to modulate microbiome function and subsequently host attributes, such as feed efficiency. In this sense, dividing the microbial community into functional groups could simplify comparative analyses of rumen communities. Functional groups could be detected, for example, by using computational approaches

Outstanding Questions

What are the major functional groups present in the rumen? Are important functional groups still missing from our analysis due to lack of isolation, characterization, and understanding?

Can metagenomic data be used to determine functional groups or is the depth of proteomic data enough to properly reflect a more realistic view of the functional potential and to provide an understanding of each functional group ecological niche?

How resilient are the described rumen community states to environmental perturbation, and what are their drivers?

What is the extent of founder effects on rumen community states in the mature rumen microbiome?

How can we modulate the course of electron flow through the rumen ecosystem from one functional group to the other?

What is the proportional role of the various inputs, outputs, or intermediate metabolites produced or consumed by functional groups in the establishment of community states and functional group interactions?

[57,58]. The approach of Levy and Borenstein [59] that regroup co-occurring metabolically competing microorganisms using *in silico* metabolic networks models [60] at the large-scale level, could be particularly suitable when no empiric information is available.

Furthermore, understanding the consequences of microbial metabolism on the generated microbial interactions may be the strongest factor in these ecosystems that could serve to perfect future analyses and modifications of rumen microbiome structure and function (see Outstanding Questions). Functional groups could also resolve taxonomy confusions owing to horizontal gene transfer events as well as functional redundancies. More broadly, identifying, modeling, and manipulating functional and ecological networks will allow us to reduce methane emissions, increase feed efficiency, and reduce antibiotic use in agriculture.

Acknowledgments

Resources
[i]www.fao.org/docrep/016/me992e/me992e.pdf
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