Contrasting Strategies: Human Eukaryotic versus Bacterial Microbiome Research

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ABSTRACT
Most discussions of human microbiome research have focused on bacterial investigations and findings. Our target is to understand how human eukaryotic microbiome research is developing, its potential distinctiveness, and how problems can be addressed. We start with an overview of the entire eukaryotic microbiome literature (578 papers), show tendencies in the human-based microbiome literature, then compare the eukaryotic field to more developed human bacterial microbiome research. We are particularly concerned with problems of interpretation that are already apparent in human bacterial microbiome research (e.g., disease causality, probiotic interventions, evolutionary claims). We show where each field converges and diverges, and what this might mean for progress in human eukaryotic microbiome research. Our analysis then makes constructive suggestions for the future of the field.

Keywords
Cross-domain interactions; eukaryotic microbiome; eukaryotic probiotics; microbiome causality; mycobiome.

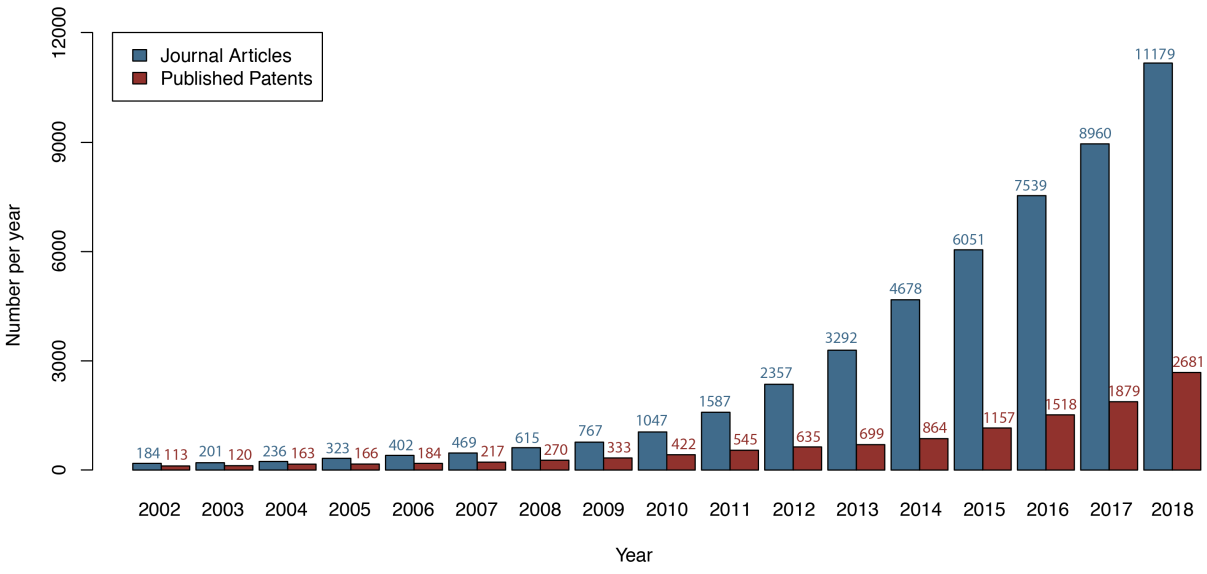
INTRODUCTION
So far, most wide-ranging discussions of human microbiome research have focused on bacterial findings to gain insight into how the field is developing. Eukaryotic microbiome research is growing rapidly but has important differences from bacterial approaches. These differences may stimulate new trajectories of research development and also suggest how to overcome challenges inherent in microbiome research. We compare human bacterial and eukaryotic microbiome literature, particularly to find out whether the interpretive challenges that exist in human bacterial microbiome research are occurring in human eukaryotic microbiome research. These problems have to do with diversity descriptions and disease associations, potential treatments, and the evolutionary and ecological status of microbiota in relation to their human hosts. Our findings suggest that these issues play out somewhat differently in human eukaryotic microbiome publications, due to basic ecological properties of eukaryotic microbiota. We conclude with reflections on whether human eukaryotic microbiome research will follow the same trajectory of development as human bacterial microbiome research.

General microbiome research trends
Anyone thinking about microbiology these days is likely to consider it as occurring within 'the Age of the Microbiome' (Suhr and Hallen-Adams 2016, p. 1057). This informal historical label describes a context in which high-throughput sequencing methods enable

culture-independent approaches to learn about microbial diversity. These investigations target an extensive range of microbial communities and environments, including those in and on host bodies. Unsurprisingly, the host-microbe relationships that have had the most scientific and popular attention involve humans, for whom health often seems to be a microbiome matter. This marriage of medical interests and microbiome methods is still in its honeymoon phase, but has contributed to quantitative leaps in the amount of microbiome literature in publication databases (Fig. 1).

Because of the potential medical and environmental applications of microbiome findings, a rising number of patent applications trail in the wake of the literature. An emerging sentiment in drug development is that 'ignoring the microbiome is not an option' (Olle 2013, p. 315). A fairly recent comparison of microbiome literature and international patents finds a fourfold increase in microbiome patents between 2004 and 2012 (Olle 2013). Our own examination of microbiome publications and patent data shows a very similar comparative growth dynamic (Fig. 1). There is a 10-fold growth of publications between 2010 and 2018. Patents, which naturally lag behind publications, show a six-fold increase between 2010 and 2018. The great majority of these patents are for diagnostics and live biotherapeutics (primarily probiotics, but also some faecal matter/microbiota transplants [FMT]).

Figure 1. Microbiota/microbiome journal articles (blue bars) and published patents (red bars) published between 2002 and 2018.
Search terms: microbiome OR microbiota OR "gut flora" OR "gut microflora". Sources: PubMed and European Patent Office ('Worldwide EN – collection of published applications in English').



Although studies of human microbiomes sometimes target viral and archaeal members of microbial communities (e.g., Minot et al. 2011; Moissl-Eichinger et al. 2018), or at least mention 'non-bacterial' taxa (e.g., Koenig et al. 2011; Yatsunenko et al. 2012; Heintz-Buschart et al. 2017), the primary focus of both the literature and patents is indeed bacteria. Viruses, Archaea, protists and other microbes have until recently been minority interests (see Rowan-Nash et al. 2019, for a comprehensive list of such studies). There are

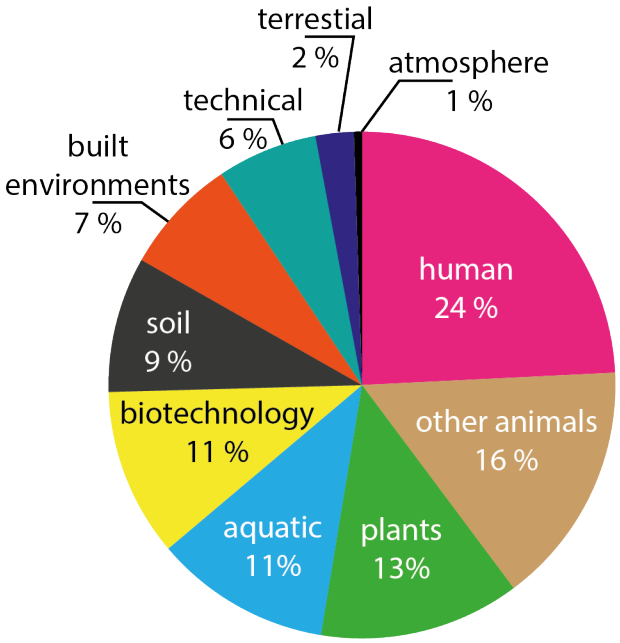
practical methodological reasons for this privileging of bacteria (i.e., sheer abundance, relevant tools, genome structure), as well as more substantive justifications. Bacterial genes vastly outnumber eukaryote genes in communities (Qin et al. 2010) and bacteria predominate in the mass production of metabolites known to have immune and other effects (Levy et al. 2016; Postler and Ghosh 2017). Conversely, the functional impact of eukaryotic microbes has primarily been detected in cases of active pathogenicity. In most human microbiome research, therefore, it was not unreasonably assumed that eukaryotic microbes would be unimportant in terms of microbial community analysis because of being too low in abundance (except for standard pathogenic outcomes for the host). Our discussion will address the growing body of eukaryote-focused microbiome research, with a focus on studies in humans, and whether there are meaningful differences between human bacterial and eukaryotic microbiome research.

Bibliometric overview of eukaryote microbiome research

Despite limited attention during the early phases of microbiome research, scrutiny of eukaryotic microbiomes has surged in recent years. In this article we define eukaryotic microbiomes as consisting of unicellular organisms, which means we exclude macroparasites such as helminths despite their important immunomodulatory interactions with human hosts (e.g., Parker and Ollerton 2013; Maizels and McSorley 2016). We present first a quantitative bibliometric sketch of the literature, and then home in on some key historical papers that have helped set the agenda for the development of human eukaryotic microbiome insights.

Using the biomarker search terms "28S OR 18S OR ITS1 OR ITS2 AND microbiome" (337 results) and a more general expression "Mycobiome OR mycobiota OR ("fungal microbiota") OR ("fungal microbiome") OR "eukaryotic microbiome" OR "eukaryotic microbiota" OR ("microeukaryotes AND sequencing")" (842 results) we found a total of 1091 articles. After manual inspection of this literature, we removed papers that were not about eukaryotic microbiome research (i.e., did not feature culture-independent community-wide molecular methods), were not in English, or were reviews (see Supplementary Methods). Of the remaining 578 papers, around 24% focused on human eukaryotic microbiomes (Fig. 2; Table S1). 'Other animals' and 'plant' eukaryotic microbiomes were the next most common focus. Rumen and arthropod microbiomes were the predominant subjects in the 'other animal' category, with occasional reference to whether insects carry human eukaryotic pathogens (e.g., Thongsripong et al. 2018). Other categories are broadly 'environmental'. These include the eukaryotic microbial communities in aquatic niches (e.g., oceans, sea sediments, hypersaline lagoons), soils (e.g., agricultural soils, biochar), terrestrial sites (e.g., caves, rocks), and human-built environments (e.g. wastewater treatment sites, mines, buildings). The last category includes a few studies that describe indoor eukaryotic microbiota and their potential impact on human health (e.g., Hanson et al. 2016; Rocchi et al. 2017). 'The biotechnology' category features studies of eukaryotic microbes detected in industrial food production and storage. Papers that focused on experimental and computational methods for analysing eukaryotic microbiomes were placed in the 'technical' category.

Figure 2. Eukaryotic microbiome niches represented in our sample of 578 data-generating papers.
We excluded review papers. For the exact numbers of papers and full definitions of categories see File S1 and Table S1.



Human microbiome sites include the gut, mouth, skin, lungs, vagina, nasal cavities, and wounds. All the main eukaryotic supergroups have been detected in these human-based investigations, even plants (Hamad et al. 2016; for discussion, see below). However, fungal microbiome ('mycobiome') papers comprise more than 80% of the total human eukaryotic microbiome papers. A 'disease-centric' focus, rather than biodiversity or health, is particularly common in human gut mycobiome research (Huseyin et al. 2017; Nash et al. 2017). Why is there so much work on the fungal microbiome? Probably because fungi are the most abundant eukaryotic microbes in the gut and thus the easiest to detect (Hamad et al. 2016). Although all eukaryotic microbiome studies in our large sample of texts analyse microbial communities with tools derived from bacterial microbiome research, these methods have had to be adapted for eukaryotic genomes and still require development (Huseyin et al. 2017; Nilsson et al. 2019).

Historical overview of human eukaryotic microbiome research

Human bacterial microbiome studies began to gain traction in the early to mid-2000s (Zoetendal et al. 2001; Eckburg et al. 2005). Although human eukaryotic microbiome research is less prominent and prolific, a number of formative studies were published not many years later. One of the earliest examined fungal microbiota from healthy and diseased (psoriatic) human skin via PCR of 18S ribosomal genes, which are biomarkers commonly used for eukaryotes (Paulino et al. 2006). The main finding was that both skin states host large numbers of *Malassezia* (a yeast).

Two gut-based eukaryotic microbiota studies followed in 2008. Both used DGGE, an older 'community fingerprinting' method, to reveal the low diversity of the eukaryotic gut microbiome (Nam et al. 2008; Scanlan and Marchesi 2008). As expected, eukaryotic diversity is far lower than bacterial diversity in the gut. Fungi and *Blastocystis* sequences were detected in these microbiomes. *Blastocystis* are obligate anaerobes in the Stramenopiles group. There are many sub-types (or even species in their own right), and not all are pathogenic.

'Mycobiome' is a term originally coined for a molecular fingerprint study of fungal communities on salt-marsh plants (Gillevet et al. 2009). In 2010 the term was first used for a

human fungal study to describe an oral fungal microbiome study carried out with ITS1 pyrosequencing (Ghannoum et al. 2010). ITS refers to internal transcribed spacers in nuclear ribosomal sequence. Both ITS1 and ITS2 have become standard molecules for amplicon (aka 'metabarcode') sequencing in fungal microbiota (Schoch et al. 2012; Blaalid et al. 2013). As well as promulgating the handy term of mycobiome (now sometimes confusingly applied to non-molecular research), Ghannoum and colleagues found more diverse and abundant fungi than expected, and that these fungi had no discernable relationship to disease. This result led to an inference of a 'baseline healthy mycobiome' (Ghannoum et al. 2010, p. 1) even though the sample size was only 20. Similar generalizations have been made in bacterial microbiome research with equally limited samples.

Another human gut study examined eukaryotic microbiomes of low birthweight infants (LaTuga et al. 2011). ITS pyrosequencing detected eukaryotes that included fungi and nematodes; bacteria were not as diverse and abundant as expected in this host context of low birthweight. There was much more environmental acquisition of microbes than predicted for one-month-old infants.

A broad 18S investigation of mammalian gut microbiota, including humans, confirmed expectations of low eukaryotic diversity (Parfrey et al. 2014). These communities included species of *Blastocystis*, *Entamoeba*, *Trichomonas*, and yeast, all of which the authors suggested were commensals rather than pathogens (see below for further discussion of pathogenicity). The study detected diet-driven changes in the composition of this eukaryotic microbiome. It also very usefully compared general findings about prokaryotic and eukaryotic microbiomes (see Table 1).

Table 1: General comparison of mammalian gut bacterial and eukaryotic microbiota (from Parfrey et al. 2014).

Bacterial microbiota	**Eukaryotic microbiota**
Consistent across samples	Patchy distribution
Very diverse	Not very diverse
Very abundant	Not very abundant
Commensal (mostly)	Commensal?

A similar comparison of bacterial versus fungal microbiome research accompanied a human gut study of the mycobiome (Hallen-Adams et al. 2015; see Table 2).

Table 2: General comparison of human gut bacterial and fungal microbiota (from Hallen-Adams et al. 2015).

Bacteria	**Fungi**
High abundance	Low abundance
Very diverse	Limited diversity
Stable	Unstable
Resident, with ecological roles	Many transients with no ecological role

On the basis of these and other formative papers, it is now fairly well established that when compared to bacteria, eukaryotic microbes in human hosts have smaller populations and less overall diversity (however, see caveats below). These factors, combined with more complex genomes and behavioural repertoires, mean eukaryotic microbes may also have different evolutionary strategies and ecological roles. Awareness of such differences indicates to at least some researchers that the field of eukaryotic microbiome research should *not*,

therefore, 'simply follow in the methodological footsteps of bacterial ecology and hope for similar success' (Keeling and del Campo 2017, p. R541).

This is the issue that motivates us. Our key question is whether eukaryotic microbiome research strategies are actually different, or whether they primarily follow bacterial microbiome approaches. Our focus is not basic methods (although we mention them), but the assumptions that drive study designs and the interpretations that are made of eukaryotic microbiome findings in human niches. We target human gut microbiome studies because of the abundance of attention given to this body site due to the systemic effects of gut processes.

Comparing bacterial and eukaryotic gut microbiome research strategies

Bacterial human microbiome research has several broad strategies: it describes taxonomic composition, finds associations rather than causes and mechanisms, often makes 'dysbiosis' claims about normal or 'imbalanced' compositions (Table 3), and might then invoke probiotics or microbiota transplants as microbiome-remediating treatments (O'Malley and Skillings 2018). We explain all these aspects as we show whether and how these features appear in eukaryotic microbiome research.

Table 3: Key concepts affecting eukaryotic microbiome interpretations.

Concept	Definition
Contextual pathogenicity	A harmful relationship that occurs only in certain environmental contexts, such as host episodes of compromised immunity.
Contextual mutualism	A beneficial relationship that occurs only in certain environmental contexts, such as when an interaction between two microorganisms leads to host benefits.
Cross-domain interactions	Interactions between organisms in different domains (e.g., bacteria and protists), especially interactions believed to be ecologically meaningful. These relationships are also called 'cross-kingdom', 'transkingdom', and 'interkingdom', although they only sometimes concern interactions between organisms in different eukaryotic kingdoms.
Dysbiosis	A purportedly negative state of microbiomes in a host, in which some sort of alteration in the microbiome correlates with some sort of illness in the host. We consider this a very problematic term in microbiome research.
Homeostasis	A purportedly positive state of microbiomes in a host, in which some sort of microbiome composition correlates with a broadly healthy state of the host. Synonyms include 'normobiosis' and 'eubiosis'. We consider all of them equally problematic for microbiome research.
Transience	The length of time any microorganism occupies the host before disappearing; usually implies short rather than lengthy residence. 'Resident' microbes are those that persist for long periods of the host lifecycle.

Diversity descriptions. Descriptive studies begin and end with a catalogue of organisms inferred from sequence. Human bacterial microbiome research began with this

descriptive aim, and eukaryotic microbiome analyses also commonly focus on diversity metrics, sometimes with very limited samples. For instance,

> 'The primary objective of this study was to evaluate the broad diversity of eukaryotes in a single fecal sample' (Hamad et al. 2012, p. 2);
>
> 'The aim of this study was to carry out a systematic molecular analysis of the diversity and composition of the intestinal fungal microbiota' (Ott et al. 2008, p. 832).

Researchers also probe for specific organisms (e.g., *Blastocystis*, *Candida*), which is again characteristic of some clinical bacterial microbiome work (e.g., Scales et al. 2014). Eukaryotic microbiome studies may home in on particular organisms such as *Blastocystis* even when they have 'unresolved clinical significance' (Stensvold et al. 2007; Scanlan 2012). *Blastocystis* function is not well understood in the gut, and its ecological roles are not clear overall, but microbiome studies may be generating additional insights (see below).

However, despite mimicking the descriptive diversity focus of bacterial microbiome research, eukaryotic microbiome analyses have already identified problems with this emphasis on composition metrics. Notably, there is not yet a full picture of how eukaryotic microbiome statistics fluctuate across different individuals and populations. Absolute abundance quantification is still in its infancy even in bacterial microbiome studies and has not yet been applied in most eukaryotic microbiome surveys. Moreover, eukaryotic microbiome studies that use universal 18S primers amplify all eukaryotic sequences, including those that originate from the host or diet. One consequence is that many fungi and other eukaryotes may be food-borne, which explains findings of plant DNA in faecal samples (e.g., Nam et al. 2008, where, curiously, many of the sequences map to ornamental flowering plants as well as food plants). Ingestion via other mechanisms (inhaling, swallowing saliva) may also occur, which means diversity statistics for eukaryotic microbiota compositions may be misleading. In other words,

> 'We must ask whether identifying the full fungal diversity [of the gut] is a meaningful goal if a majority of the fungi are "passing through", and their contribution to the gut ecology is likely to be minimal' (Suhr et al. 2016, p. 212).

A recent study that manipulated diet to exclude fungal sources concluded that 'fungi do not routinely colonize the GI [gastrointestinal] tracts of healthy adults' (Auchtung et al. 2018, p. 1). If in fact mycobiome and other eukaryotic microbiome studies are revealing 'illusory' diversity (Hallen-Adams and Suhr 2017; Huseyin et al. 2017), what does it imply for the interpretations made of eukaryotic microbiome findings so far? In human bacterial microbiome research, there has been limited attention as to whether organisms are resident or transient (Table 3). A few interesting exceptions include David et al. (2014), who detected many food-borne bacteria and fungi (some of which expressed genes), and Minot et al. (2011), who took transience into account for viruses in the gut. It is now better recognized that numerous insects do not have resident bacterial microbiota, and that this has important implications for bacterial community stability, host fitness and mutualism (Hammer et al. 2017, 2018; Ross et al. 2018). Recognizing this issue earlier in the development of eukaryotic microbiome research may allow more fine-grained ecological and evolutionary insight into microbiota dynamics. New approaches for decreasing contamination in eukaryote samples and thus removing probable transients include, for example, restriction enzyme digestion (Flaherty et al. 2018). In addition, the low diversity and potential transience of eukaryote

microbiota may inspire better functional studies to assess whether transient and resident microbes have important physiological effects on the host.

On top of persistence issues, there are also some very interesting distribution patterns in eukaryotic microbiomes that call out for explanation. In bacterial microbiome succession, as humans mature from infant to adult, there is believed to be a fairly straightforward pattern of more organisms, more diversity, and more stability over time (Koenig et al. 2011; Costello et al. 2012). But if there really are 'no clear successional patterns' in the eukaryotic microbiomes of infants (Wampach et al. 2017: 14; Ward et al. 2018), what are the implications? Might the apparent variability and low stability of eukaryotic microbiota patterns indicate different ecological processes, or are such findings merely artefacts of small populations, limited sampling, and transience? There are no answers yet to such questions, but they immediately indicate that careful ecological analyses are required to understand eukaryotic microbiome patterns. We consider this an asset rather than a liability for the field and elaborate further on this point below.

From diversity to disease associations. Bacterial microbiome research has very commonly made a move from diversity analysis to establishing disease associations (e.g., between phyla proportions and obesity). Eukaryotic microbiome researchers have also seen the attractions of identifying such connections. For example,

> 'Future studies of microbial eukaryote communities should focus more on identifying variation that is associated with different phenotypic states, including disease states' (Parfrey et al. 2014, p. 10).

A standard claim in human bacterial microbiome research is that lower diversity is associated with illness (e.g., Kostic et al. 2015; Sonnenburg et al. 2016). Taking this relationship as a background assumption has now been criticized on empirical and theoretical grounds (Shade 2017), with an increasing number of findings connecting high diversity to disease (e.g., Anahtar et al. 2015; Jiang et al. 2015; Duvallet et al. 2017). Nevertheless, many such connections continue to be made in human bacterial microbiome studies, especially medically oriented ones.

Early in eukaryotic microbiome research, this notion of low diversity typically having associations with disease was challenged. Ott et al. (2008) found that *increased* fungal diversity is associated with illness. Other findings of this sort lead eukaryote microbiome researchers to caution that:

> 'Drawing a generalized conclusion about the correlation between disease and fungal diversity is … difficult' (Cui et al. 2013, p. 7).

Despite such warnings, human eukaryotic microbiome research now increasingly makes connections between diversity and host health, and may even argue for a 'colonization deficit' of eukaryotic microbes in Western populations (e.g., Laforest-Lapointe and Arrieta 2018). This has been an influential claim in human bacterial microbiome research (e.g., Sonnenburg et al. 2016). Even though attempts to identify a 'core microbiome' and a 'healthy baseline microbiome' have so far failed in bacterial microbome research (Lloyd-Price et al. 2016), such states are now being eagerly sought in eukaryotic microbiome studies, especially fungal ones (Wheeler et al. 2016; e.g., Nash et al. 2017). Might it be more feasible to achieve such baselines with fewer taxa and lower abundance in eukaryotic microbiota? Statistically, this seems unlikely, especially if the majority of detected fungi and other eukaryotes are not colonizing the gut and merely passing through.

From association to causation? Bacterial microbiome research is currently reaping the harvest of a slew of study design problems. Small sample sizes mean studies are statistically underpowered. The association/correlation focus means that there is little that can be said about causation. Prediction is also limited, partly because of sample size and partly because of unknown causal structure. Genuine explanation of cause-effect relationships is unlikely without mechanisms, which if articulated at all tend to be highly speculative. Finally, ecological modelling is still a minority achievement in the vastness of human bacterial microbiome literature.

Eukaryotic microbiome research is also afflicted by similar problems on a smaller scale. Limited samples abound (<10 in some studies), as do associations from which predictions cannot be generated reliably. There are modest attempts at causal explanation and ecological modelling. Although we see some divergence from the trajectory of bacterial microbiome research (see below), the aspirations of many human eukaryotic microbiome researchers are to follow the trail blazed by bacterial microbiome research.

One good illustration of why this strategy is not likely to end well comes from connections made between obesity and bacterial microbiota. Many efforts were made to find specific compositions that would determine obesity in human hosts. The same approach has also been taken for the eukaryote members of those communities. For example, a study of 52 humans found that:

> 'Obese patients could be discriminated by their specific fungal composition, which also distinguished metabolically "healthy" from "unhealthy" obesity' (Mar Rodríguez et al. 2015, p. 1).

However, there are major problems now identified in bacterial microbiome findings about obesity. Despite early promising findings of clear associations between weight and bacterial microbiome composition (Ley et al. 2006), and transfer of phenotype via microbiota (e.g., Turnbaugh et al. 2006), meta-analysis shows that phyla supposedly associated with obesity correlate with weight increase *or* decrease, and that experimental replications of transfer-of-phenotype studies are contradictory (Fleissner et al. 2010; Harley and Karp 2012; Walters et al. 2014; Sze and Schloss 2016). There is a loss of effect due to sample sizes being too small, plus dietary confounders in the experiments that obscure causal pathways and their directionality. Following this approach to try to establish causal relationships might not be the best way to develop eukaryotic microbiome investigations of obesity and other conditions. Moreover, bacterial microbiome research has additional problems that accompany health/illness association claims.

'Dysbiosis' interpretations. 'Dysbiosis' is a very common term in bacterial microbiome research, where it vaguely describes a microbiome with any kind of variation that might be associated with illness (see Hooks and O'Malley 2017; Table 3). This loose, circular use of the term has many problems. First and foremost, even if dysbiosis exists, it is not known whether it is a cause or consequence of the disease. If it is somehow causal, is it whole-community causation or are there just some key causal members of the microbiota? At best, dysbiosis is a suggestion that something may have changed in the microbiota, and that this change might be connected to the disease; at worst dysbiosis 'obscures mechanisms' (Olesen and Alm 2016). Dysbiosis claims usually say nothing about function, and they talk about balance without quantification. 'Normal' microbiome compositions are asserted as the non-dysbiotic state, but there is no way to index 'normality' except in contrast to compositions associated with extremely disease-specific conditions. Illness-associated

microbiome indices at best confirm diagnoses of disease (Gevers et al. 2017; Hooks and O'Malley 2017).

There is already considerable discussion of 'dysbiosis' in human eukaryotic microbiome research (around 8% of the papers in 578 papers analysed by total text, versus 5% of total bacterial microbiome papers analysed via abstracts only – see (Hooks and O'Malley 2017)). The term is particularly popular in fungal microbiome studies (see Table 4).

Table 4. Representative dysbiosis quotes from eukaryotic microbiome work

Citation	Quote
Mar Rodríguez et al. 2015, p. 2	'Mycobiome dysbiosis is relevant in inflammatory diseases' and occurs when the 'finely tuned equilibrium between the host and microbiota [is] disrupted'
Sokol et al. 2017, p. 1039	'The faecal fungal mycobiota is imbalanced in patients with IBD … clear fungal dysbiosis'
Li et al. 2014, p. 958	'Gut bacterial dysbiosis [that is] induced by antibiotic therapy could cause fungal overgrowth'
Lewis et al. 2015: 498	'The dysbiosis of Crohn's disease extends beyond bacteria to include fungi'
Iliev and Leonardi 2017, p. 1	'Fungal dysbiosis [in Crohn's Disease] is characterized by an increased load of fungi … with pro-inflammatory effects, and a decrease in fungi with beneficial effects'
Iliev and Leonardi 2017, p. 1	'Dysbiosis … is widely used to describe altered bacterial communities as both a cause and a consequence … A similar process involving fungal communities – fungal dysbiosis --- could affect the host mycobiota'

We draw attention to the last quote in Table 4, which says dysbiosis can be a cause and a consequence. Normally, we expect causes to produce effects (even though effects can be part of a positive feedback loop, sequential causal steps can still be specified). But in most cases when dysbiosis is mentioned in eukaryotic (and bacterial) microbiome research, nobody has a clue what the cause or effect is (Arrieta et al. 2018). Most dysbiosis statements are bet-hedging, as captured by the quote in Table 4: 'dysbiosis' might be a cause, but then again it might be a consequence. Usually, if we find something is a cause *and* a consequence, we know there is a problem. It is probably too late for any field of microbiome research to reverse this compulsion to discuss findings in terms of dysbiosis, but perhaps there is still a chance that eukaryotic microbiome research can be more careful about how the term is applied.

Eukaryotic microbiota treatments: probiotics and FMTs. If dysbiosis exists, so apparently must its opposite, often called 'homeostasis' (Table 3). What can be done to turn a supposedly dysbiotic microbiota into a hypothetically homeostatic one? In most bacterial microbiome research in medical contexts, probiotic interventions and faecal microbiota/matter transplants (FMTs) are the main 'microbiome' treatment. It is often suggested that bacterial probiotics such as lactobacilli or bifidobacteria can revert the microbiota to a healthier state for the human host (e.g., Rauch and Lynch 2012; Walsh et al.

2014; Libertucci and Young 2019). Many contested claims are made about probiotics, and despite some consistent findings in mouse studies, human probiotic meta-analyses and systematic reviews are at best 'ambiguous' about probiotic benefits for most illnesses (see Marchesi et al. 2016; Suez et al. 2019). Nevertheless, the majority of so-called bacterial 'microbiome' patents are for probiotics (Hooks et al. 2018; Supplementary Material). Does this trend have any parallels in eukaryotic microbiome research?

Eukaryotic probiotics are less well-known, and those for humans are less diverse than bacterial probiotics. The main eukaryotic probiotic is a strain of *Saccharomyces cerevisiae* (Brewer's yeast), called *boulardii* (often called *S. boulardii* rather than the correct nomenclature of *S. cerevisiae* var. *boulardii*). This yeast is widely believed to have positive effects on gut health, especially against diarrhoea, and numerous studies attest to this therapeutic association (e.g., Czerucka et al. 2007; McFarland 2010). As well as *S.* var *boulardii*, some other microbial fungi (e.g., *Aspergillus oryzae*, *A. niger*, *Candida pintolepesii*) have been used as livestock probiotics and their use is associated with weight gain (Simon et al. 2001; Lara-Flores et al. 2003; Kabir et al. 2004; Chaucheyras-Durand and Durand 2010). However, little that has been learned from other animal probiotic studies translates well to humans and vice-versa. In mice experiments, for example, *S.* var *boulardii* results in weight loss and has even been touted as an anti-obesity agent (Everard et al. 2014). To add further complications, *C. albicans*, the label for what is probably a species complex with variable health implications (Criseo et al. 2015), has in humans have been linked to gut disease and mental health disorders (Severance et al. 2017; e.g., Sovran et al. 2018), as well as poor outcomes for FMT treatments (Zuo et al. 2018). But in mice, *C. albicans* (not normally a mouse gut colonizer unless mice have received antibiotic treatment) has been interpreted as 'protective' and preventive of intestinal diseases and viral infections (Wheeler et al. 2016; Jiang et al. 2017; Tso et al. 2018).

Translating from rodent studies to human implications is already problematic in bacterial microbiome research (Nguyen et al. 2015) but should not be unexpected. Parasitology recognizes many examples of organisms eliciting different immune responses in different hosts, such as the contrasting effects of the tapeworm, *Hymenolepis diminuta*, in rats and mice (McKay 2010). More broadly, there are also highly variable interactions between helminths and bacterial microbiota just in human intestines (Cortés et al. 2019), and explaining such variability is of considerable relevance for treatments that might be based on eukaryotic microbiota.

Probiotics, both bacterial and eukaryotic, are often assumed to have their effects by colonizing the gut, then modifying the microbiota and what it is doing to the host (Barc et al. 2008; e.g., Rauch and Lynch 2012; Everard et al. 2014). However, gut colonization of bacterial probiotics is not uniform in humans, and mice seem not to be colonized at all (Zmora et al. 2018). This is also the case for eukaryote probiotics. Effects are strain-specific and rarely generalize to other strains in the same species (McFarland 2010; Vanhee et al. 2010). Furthermore, when a probiotic such as *S.* var *boulardii* does not remain long in the normal gut (Edwards-Ingram et al. 2007; Vanhee et al. 2010), this raises questions about the extent of its interactions with bacteria and host in the yeast's short transit times. There may be direct effects from the yeast on the gut, although very little is known about mechanisms, especially in organisms other than mice; in addition, any response to probiotics is highly individualized (Simon et al. 2001). Although colonization can be improved by giving patients antibiotics, *S.* var *boulardii* is often used in the first place to treat patients who were already suffering from antibiotic-associated diarrhoea (Hempel et al. 2012).

We have written elsewhere about the oversimplifications in assuming that bacterial probiotics are reconstructing the microbiota (Hooks et al. 2018; Lynch et al. 2019). So far,

the probiotic treatment aspect of microbiota interventions has not gained a great deal of traction in eukaryotic microbiome research. It will. We think that is simply inevitable, given ease of treatment, low risk, and commercial pressures. Using bacterial probiotics to intervene in bodily and mental health conditions is already a major growth industry (Fig. 1), albeit with many limitations (Hooks et al. 2018). Eukaryotic microbiome researchers should even now be thinking of how to do their probiotic analyses in more sophisticated ways. These must inevitably involve functional studies that look at the direct effects and mechanisms of putatively probiotic eukaryotic microbes in the gut, and take human and environmental variation into account (Cortés et al. 2019). Although we have focused on unicellular eukaryotes, we recognize the growing potential for informing microbiome research and therapies with ongoing efforts to develop helminth-based microbiome treatments (e.g., Lukeš et al. 2014; Rapin and Harris 2018) as well as the potential for problems that can arise from such therapies (McKay 2015).

FMTs are the other main intervention and treatment in human bacterial microbiome research. Although there are no exclusively eukaryotic microbiota transplants (single or a few microbes introduced into a host are better described as probiotics), there has been a little attention to how eukaryotic microbes work in FMT treatments. Some of the existing scrutiny is negative: if *Blastocystis* and other protists are found in donor stools for FMTs, that stool sample is often excluded from the treatment (Stensvold and van der Giezen 2018). Fungal contributions to general FMTs are just beginning to be assessed. One recent study found intriguing relationships between the bacteria and fungi in FMTs, with different correlations between fungi and bacteria apparently having different therapeutic effects on hosts (Zuo et al. 2018). 'Cross-kingdom' interactions in general are an emerging and very promising emphasis in eukaryotic microbiome literature, and may indicate a fruitful divergence point from existing trends in bacterial microbiome research.

Cross-domain relationships and their implications

Despite the very basic descriptive focus of most eukaryotic microbiome studies so far, some interesting findings are already emerging about so-called cross-kingdom relationships (aka 'interkingdom' or 'transkingdom'; see Table 3). These labels usually refer to interactions between different *domains* of life – see (Rowan-Nash et al. 2019) and we will refer to them as such. This attention to eukaryotic-prokaryotic-viral interplay (or the potential of it) is at least partly due to the low abundance of eukaryotic microorganisms, with mass effects not being expected (unlike in bacterial microbiome research). Researchers instead have to probe more deeply for hypotheses about the feasible effects of eukaryotic microbes on the host, both directly but increasingly indirectly, as a result of interactions within the entire microbiota between eukaryotes, prokaryotes and viruses.

In human studies, a range of relationships has been investigated in order to understand the pathways by which eukaryotic members of the microbiota might affect the host. Fungi and bacteria have long been known to interact, especially in oral niches (Shirtliff et al. 2009), and microbiome methods are also illuminating gut relationships between domains. For example, *Methanobrevibacter* (a genus of Archaea) and *Candida* were found to correlate positively in the gut shortly after the host eats large amounts of carbohydrate, and negatively in a high-fat-protein diet (Hoffmann et al. 2013). However, *Candida* in the gut may be a contaminant or transient from the mouth, which ties in with the rapid response to carbohydrate consumption (Auchtung et al. 2018). Other studies have detected simple correlations between bacteria, fungi, inflammation and disease (e.g., Lewis et al. 2015; Fujimura et al. 2016).

Associations have been found between *Blastocystis* and other protists with proportions of bacterial phyla, although these patterns have sometimes been unhelpfully described in terms of 'dysbiosis' or non-dysbiosis (e.g., Audebert et al. 2016). Bacterial increases and decreases are thought in some conditions to be driven by the protists (Nieves-Ramírez et al. 2018), but not necessarily with any implications for host health (Andersen and Stensvold 2016). *Entamoeba* presence also correlates with bacterial phyla proportions, and may explain increases in putatively anti-inflammatory bacteria (Morton et al. 2015). Interactions between fungi and bacteria in the intestines of mice on high-fat diets are proposed as contributors to obesity (Heisel et al. 2017). To add to the diversity of such cross-domain relationships, some studies have inferred interactions between gut metazoans and eukaryotic microbes that affect host health (e.g., Reynolds et al. 2015; Chabé et al. 2017). So far, however, most work on eukaryotic viruses and their role in interdomain interactions in the human gut have focused on the direct impact of these viruses on the host (Norman et al. 2014), rather than their effects on microeukaryotes colonizing the gut. This is an ecological gap that needs filling because of the potential implications of viral infections for the relationship of eukaryotic microbes to the human hosts.

Although some cross-domain research aims to gain closer insight into apparently pathogenic protists (e.g., *Giardia*) and their relationships with bacterial microbiota in producing host disease (Barash et al. 2017), many studies explore more positive interactions. In many such cross-domain analyses, distribution patterns are used to formulate hypotheses about the evolved nature of these interactions, such as the lower abundances of fungi being seen as a 'reservoir' for certain functional interactions in particular contexts (Huffnagle and Noverr 2013). However, it is important to recognize that when cross-domain correlations are found, these are not a sufficient basis on which to infer 'interactions', even if some studies suggest this to be the case. Transience could be a major factor influencing any effects on the host. If an organism is present only briefly, effects are more likely to be pathogenic (Lukeš et al. 2015). Even if organisms have a persistent presence, other factors and causal structure may be involved, meaning the relationships may be indirect or even artefactual. For example, Hoarau et al. (Hoarau et al. 2016, p. 1) say, 'we defined the microbial interactions [between fungi and bacteria] leading to dysbiosis'. Leaving aside 'dysbiosis', what they found were correlations at the sequence level, and physical proximity at the microscopic level. These factors correlate with a host state of illness. But knowledge of actual 'interactions' requires causal evidence and mechanisms that capture what the organisms are actually doing. What often happens instead are vague gestures toward these mechanisms, some of which are more plausible than others (Table 5).

Table 5. How do eukaryotes interact with bacteria in gut microbiota?

Citation	Quote
Chabé et al. 2017, p. 932	There is a 'need to untangle whether bacteria community structure and function impact *Blastocystis* or *Entamoeba* colonization or vice versa'
Pandey et al. 2012, p. 224	'Some of the diseases could be the outcome of predation of beneficial bacteria by *Blastocystis'*
Bär et al. 2015, p. 1	Parasitic protozoan infections 'are often accompanied by an imbalanced [bacterial] microbiota and … these bacteria may contribute synergistically to disease progression'
Sokol et al. 2017, p. 1046	'A balance [is worked out] between bacteria and a fungal microbiota' after antibiotics

Ghannoum 2016, p. 1	'Bacteria and fungi coexist in different body sites … and have evolved to cooperate in a way that is beneficial to their existence and detrimental, in some cases, to the host'

Causal relationships are clearly some way off as the sample of quotes in Table 5 indicates. But even though this is understandably the situation for a fairly new area of research, it does not license strong assumptions about mechanisms. For example, 'balance' is a problematic notion, as we noted above when discussing dysbiosis. Saying more or fewer taxa are associated with disease is not explanatory of any relationship between those taxa. And references to 'cooperation' in the absence of fitness calculations might misinterpret highly manipulative relationships (Coyte et al. 2015). It is probably better not to characterize these relationships initially with untested theoretical terms, and instead, to assess the positive and negative dynamics between the microorganismal groups, and then to evaluate the potential relationships with the host.

For example, some intra-microbiome cross-domain relationships may be simply structured, such as that between *Blastocystis* and *Ruminococcus* bacteria. The former predates on the latter, but this in turn leads to greater bacterial microbiome diversity and evenness (Nieves-Ramírez et al. 2018). Correlations between *Blastocystis* and anaerobic bacteria can also be driven by oxygen levels (Stensvold and van der Giezen 2018) and require particular age-related diversity conditions in the bacterial gut microbiota (Scanlan et al. 2018). But even though specific eukaryotic and prokaryotic community structure can lead to more anti-inflammatory byproducts, it would be a mistake to describe this as any kind of 'cooperation' or 'balance' with the host. It is a basic ecological explanation of community structure that is not straightforwardly providing benefit to any participant.

A good example of such complexities occurs with *C. albicans*, which is frequently considered an opportunistic pathogen that can harm immunocompromised hosts. However, recent research on the interactions between *C. albicans* and bacterial microbiota suggest there may be benefits to the host from the presence of *Candida*. *Candida* reductions after antifungal treatment are accompanied by bacterial depletion and more colitis in the host (Wheeler et al. 2016). In the authors' interpretation of this finding, 'fungal and bacterial communities are co-dependent and … disruption of one community affects the other' (Wheeler et al. 2016, p. 868). This type of dependence has been interpreted in other research as having benefits for the host. Jiang et al. (2017) reveal the 'protective' effects of *C. albicans* and *S. cerevisiae*, which, argue the authors, occur when these fungi substitute functionally for bacteria that have been depleted in mice intestines by antibiotics.

Interesting as this finding is, it is not clear that fungi should be conceptualized as functional replacements for bacteria. The apparent benefits of fungi can be explained somewhat differently. Tso et al. (2018) found that mice with an 'intact' or 'unperturbed' microbiota (i.e., not antibiotic-treated; the actual community composition was not analysed) were able to control *C. albicans* growth so that it lived as a commensal and did not take its pathogenic form (with hyphae). In the process, exposure to *C. albicans* conferred resistance on the mice to other pathogens (Tso et al. 2018). The authors saw this experimentally evolved process as the beginning of a mutualistic relationship, which is an interpretation that requires more careful evolutionary-theoretic consideration.

Evolving mutualistic microbiota interactions. An underlying assumption of much medically oriented bacterial microbiome research is that there is a coevolved mutualistic relationship between human hosts and microbiota. For instance,

> 'Coadaptation and mutual benefit are key features of these symbioses between hosts and their microbial communities, or microbiotas' (Relman 2015, p. 1127);
>
> 'The shared evolutionary fate of humans and their symbiotic bacteria has selected for mutualistic interactions that are essential for human health, and ecological or genetic changes that uncouple this shared fate can result in disease' (Dethlefsen et al. 2007, p. 811);
>
> 'The microbiome … has coevolved with the host for an optimal mutualism in performing crucial functions' (Viaud et al. 2014, p. 4217).

There are a number of problematic ideas about evolution and bacterial microbiota tied up in such claims. Coevolution, which requires evidence of reciprocal adaptation, has not yet been demonstrated on a broad scale in bacterial microbiota (Foster et al. 2017). In particular, the idea of a global evolved function of human bacterial microbiota (i.e., maintaining human health) is very unlikely. The same evidence for an evolved mutualistic outcome is also the evidence for the existence of so-called dysbiotic relationships: simply that there are bacteria and that they coexist with the host, sometimes in states of evolved co-dependence.

To infer mutualistic relationships (equivalent to saying there are fitness benefits on both sides despite costs), it is necessary to break interactions down to specific microbe-host pairs in order to calculate benefits (Bronstein 2001; Mushegian and Ebert 2016; Hillesland 2018). Detailed data is necessary to evaluate such relationships. If mutualisms are indeed found, mechanisms need to be sought. Cost-free byproduct mutualisms will probably occur more frequently than costly cooperative interactions. In many cases, intense intra-microbiota competition and exploitative host control are better broad descriptions of these relationships than cooperation (Foster et al. 2017). Often, bacterial microbiome researchers invoke 'stability' in the same breath as 'mutualism' (e.g., Dethlefsen et al. 2007). But again, microbiota stability is very likely to be driven by highly competitive relationships between the microorganisms, and between them and the host, rather than cooperation (Coyte et al. 2015).

In eukaryotic microbiome research, some similarly loose ideas about mutualism are already gaining ground. There are broad claims about general functions or benefits for hosts from eukaryotic microbiota. For example,

> 'Some intestinal protozoans could play an important, yet largely unrecognized, role in shaping the gut bacterial microbiota and in maintaining the host-microbe equilibrium, and they should be considered as "friends" of the human gut' (Chabé et al. 2017, p. 927);
>
> 'Many common eukaryotic residents of the human gut are commensal or beneficial rather than parasitic … Is the eukaryome [eukaryotic microbiome] beneficial overall? We do not know, and clear-cut cases of beneficial eukaryotes in the human gut are few. Yet, new findings in diverse fields suggest that we may ignore possible beneficial roles of the eukaryome at our peril' (Lukeš et al. 2015, p. 3);

Perhaps, but we may also overestimate the beneficial roles of eukaryotic microbiota 'at our peril', especially when genuine colonization is unclear, let alone strong functional effects with evolutionary implications. Should we expect mutualistic interactions between eukaryote microbiota and human hosts? So far, little evidence is available to enable the

evaluation of mutualistic relationships between humans and eukaryotic microbes (Huffnagle and Noverr 2013; Bär et al. 2015). In some cases when mutualism is posited, the relevant organism both benefits and harms. For example, in mice intestines the protist *Tritrichomonas musculis* (a parabasalid) increases host immune responses to pathogens while making the mice more prone to colitis and cancer (Chudnovskiy et al. 2016). And on the other hand, there are analyses showing potentially positive interactions between a range of microbial groups that are disadvantageous for the host (e.g., Ghannoum 2016; Liguori et al. 2016). Not only may host-directed mutualism and cooperation not be the most appropriate expectation of host-microbiota relationships, but also, any co-dependencies may change radically over time and context. 'Conditional mutualism' (Table 3) occurs when there are fluctuating benefits that are dependent on context (Cushman and Whitham 1989; Bronstein 1994), and this might be a more appropriate term for any benefit-producing relationships between eukaryotic and bacterial microbiota and hosts. But if this term applies, so will its opposite.

Contextual pathogenicity. Bacterial microbiome research is just beginning to work with the well-known idea that pathogenicity is contextual (Table 3) rather than the other side of a 'fundamental dichotomy' between commensality and pathogenicity (see Jiang et al. 2017, p. 814)). In other words, organisms are not by their nature pathogens, but their pathogenic effects are determined by context (e.g., Chen et al. 2018). Protistology, the study of eukaryotic microbes, has long recognized opportunistic pathogenicity, when organisms may live asymptomatically in a host and only cause problems when the host (or resident microbiota) health state changes (e.g., Richardson 1991; Kaplan et al. 2000). Numerous individual protists and fungi exhibit opportunism that fluctuates from fully and partly asymptomatic infections to varying levels of pathogenicity: *Dientamoeba*, *Candida*, *Entamoeba*, *Giardia*, *Toxoplasma*, *Pneumocystis*, *Blastocystis* and *Cryptosporidium* (Bruijnesteijn van Coppenraet et al. 2015; Bartelt and Platts-Mills 2016; Nieves-Ramírez et al. 2018; Bouzid et al. 2013; Osman et al. 2016). *S.* var *boulardii*, although often characterized as a probiotic and thus assumed to have a positive influence on the gut, is also an opportunistic pathogen (Edwards-Ingram et al. 2007). When patients are immune-deficient, the yeast can cause tissue and blood infections that may be life-threatening (Boyle et al. 2006). But on the other hand, *Blastocystis* may not deserve its standard status as potentially pathogenic: a detailed analysis shows it is very probably an ordinary commensal, inclined to healthy gut conditions (Beghini et al. 2017).

Although much more mechanistic detail is needed about how 'opportunism' of this sort works, eukaryotic microbiome research is already primed – with its protistology and mycology roots – to develop better experimental insights (Kaneshiro and Dei-Cas 2009). Defining the contexts of pathogenicity and the host-microorganism interactions that produce them is a finer grained way in which to generate causal insight than lumping the whole microbiota into 'dysbiotic' or 'homeostatic' categories, with mutualism assumed as the default relationship. Existing insights about the contextual nature of protist and fungal pathogenicity can serve eukaryotic microbiome research well, especially in light of this young field's growing attention to cross-domain interactions.

CONCLUSIONS AND PERSPECTIVES

What should we say to summarize the state of eukaryotic microbiome research vis-à-vis bacterial microbiome research? There are negatives and positives. Most obviously, much more methodological development is still needed. More quantified functional analysis is necessary (not just barcodes and OTUs), and more experimental work. The other area requiring development is ecological modelling. We see considerable promise in that strand of potential development. In fact, we suggest that our overview of current human eukaryotic

microbiome research shows extraordinary opportunities for more sophisticated analyses that combine ecological theory and quantitative data. Why is this?

The answer lies partly in the fact that there is so much less diversity in eukaryotic microbiomes compared to the 'overwhelming diversity' of bacterial microbiomes. The more complex the community, the more difficult it is to study without shortcuts (e.g., 'dysbiosis') and being drowned in data. This low diversity enables a strong focus on interactions between various eukaryotes and bacteria, viruses, and host conditions. Having this interactive emphasis makes an excellent basis for ecological modelling that elucidates community structure and function.

Traditionally, human eukaryotic microbes have been modelled in mice to understand disease effects. This approach is not without problems. As is the case for bacterial taxa, there are limited fungal taxa that are the same in the mouse and human gut (Richard et al. 2015). Colonizing mice with human eukaryotic microbes often requires antibiotic treatment, which although confounding the analysis of cross-domain interactions, does allow insight into mechanisms of colonization and pathogenesis (e.g., Iliev et al. 2012; Chudnovskiy et al. 2016; Watanabe et al. 2017). Germ-free mice are also used as models for eukaryotic microbes (e.g., Phillips and Balish 1966; Westwater et al. 2007), but unlike bacterial microbiome research are not yet a mainstay of causal investigation in eukaryotic microbiome research. It is not clear that germ-free models are the most effective means by which to understand the relationships between hosts and eukaryotic microbes (Naglik et al. 2008), largely because the effects on the host of these bigger but less abundant organisms have a high likelihood of being bacterially mediated.

Despite an existing platform of experimental work on human-associated eukaryotic microbes, because of interactions with bacteria (and viruses), and the abundance of these entities, a great deal of bioinformatic analysis is still needed to pick out relationships worth investigating across time and in different contexts. Computational and mathematical modelling then comes to the fore in understanding these interactions mechanistically. Bacterial microbiome research has begun illuminating potential causal interactions with computational models based on sequence data (e.g., Manor et al. 2014); such data can be used to test ecological models of community structure (e.g., Stein et al. 2013). Eukaryotic microbiome research is not at present invested in such methods, but piggy-backing on existing bacterial work by adding eukaryotic variables would valuably expand such efforts.

Eukaryotic microbiome research would also benefit from intermediate methods that fall between the complex communities described by detailed microbiome analyses and more abstract mathematical and computational models. Synthetic microbial communities are another tool being developed for bacterial microbiome research in order to understand microbial communities from the 'bottom up' (De Roy et al. 2014; Dolinšek et al. 2016; Vega and Gore 2018; Elzinga et al. 2019). Representative synthetic communities can be used to isolate hypothesized organizational and causal relationships (e.g., Venturelli et al. 2018). Even if these interactions occur naturally in much more complex communities, such simplifications nonetheless allow the identification of relevant causal variables, and the direction of causality. Although this approach is not discussed explicitly yet in eukaryotic microbiome research, it has been used to demonstrate, for example, fungal-bacterial interactions in vitro (Shi et al. 2017). Again, the lower diversity and abundance of eukaryotic microbes makes them highly amenable to such modelling, as does the general ecological focus on interactions that drives much eukaryotic microbiome research (even in medical contexts). In other words, low abundance, potential transience, instability, and lack of mass effects (see Tables 1 and 2) are highly advantageous to a more functional, explanatory and sophisticated approach in eukaryotic microbiome research. To encourage such developments,

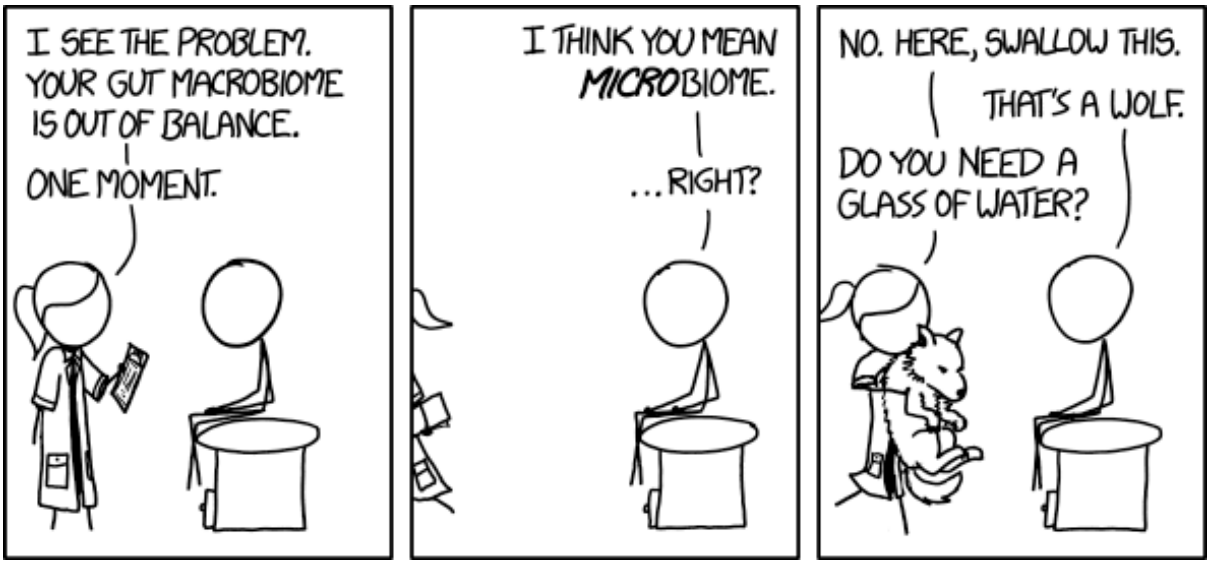
both positive and negative lessons can be learned from reflections such as ours above on the record of bacterial microbiome research.

One final point of comparison between eukaryotic and bacterial microbiome research still needs addressing. Should researchers worry about 'overselling' the eukaryotic microbiome? Some dangerously simplistic messages have emerged from bacterial microbiome research (see Eisen 2017; Hooks et al. 2018). These strong claims have broad treatment implications that include dietary interventions and probiotics. Does the equivalent exist in eukaryotic microbiome research? There are numerous statements about mycobiomes and human health benefits, but so far, less emphasis on easily implemented changes to the eukaryotic microbiome. The growing attention to cross-domain interactions may protect the field from simplistic assumptions about causal relationships, and this in turn may prevent overblown publicity as eukaryotic microbiome research develops.

Our final suggestion is that the label of 'eukaryome' or 'eukaryotome' for eukaryotic microbiomes might be worth avoiding along with oversimplification and hyperbole. Some fungal microbiome researchers encourage the uptake of the term 'mycobiome' in order to distinguish such studies from bacterial microbiome research (e.g., Cui et al. 2013). However, this term is frequently used nowadays without any actual 'microbiome' methods, simply in reference to fungal communities. Protist microbiome researchers have suggested 'eukaryome' and 'eukaryotome' as an alternative that would include helminths (Andersen et al. 2013; e.g., Lukeš et al. 2015). 'Meiofauna' is another term proposed for eukaryotic components of microbiota (e.g., Stappenbeck and Virgin 2016), even though this term has traditionally been used for organisms with sizes that fall between microbial and macrobial.

As we noted in the introduction, we excluded helminth literature from our survey purely for size reasons, because we think there are advantages to focusing on the unicellular occupants of human bodies and other hosts (e.g., shared knowledge deficits, methodological synergy). Including too many eukaryotes (especially the larger ones) might also confuse public communication about the aims and achievements of microbiome research (e.g., Fig. 3). Given the potential of eukaryotic microbiome research to clarify general microbiome strategies, introducing further obscuration would be counterproductive. Nevertheless, we acknowledge that eukaryotic microbiome research is ideally located for drawing on knowledge and strategies from macroeukaryote parasitology as well as prokaryotic microbiome and virome research. In this respect, we foresee eukaryotic microbiome research going beyond its own domain to achieve deeper insight into the relationships between human hosts and their multiscale symbionts.

Figure 3. Potential (tongue-in-cheek) problems of 'eukaryome' as a new microbiome term for eukaryotic microbiota.
Courtesy of xkcd (Munroe 2015), under CC BY-NC 2.5 licence.



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LITERATURE CITED

Anahtar M. N., Byrne E. H., Doherty K. E., Bowman B. A., Yamamoto H. S., Soumillon M., Padavattan N., Ismail N., Moodley A., Sabatini M. E., Ghebremichael M. S., Nusbaum C., Huttenhower C., Virgin H. W., Ndung'u T., Dong K. L., Walker B. D., Fichorova R. N. & Kwon D. S. 2015. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity*, 42:965-76. doi: 10.1016/j.immuni.2015.04.019

Andersen L. O. & Stensvold C. R. 2016. *Blastocystis* in Health and Disease: Are We Moving from a Clinical to a Public Health Perspective? *J. Clin. Microbiol.*, 54:524-8. doi: 10.1128/JCM.02520-15

Andersen L. O., Vedel Nielsen H. & Stensvold C. R. 2013. Waiting for the human intestinal Eukaryotome. *ISME J.*, 7:1253-1255. doi: 10.1038/ismej.2013.21

Arrieta M.-C., Arévalo A., Stiemsma L., Dimitriu P., Chico M. E., Loor S., Vaca M., Boutin R. C. T., Morien E., Jin M., Turvey S. E., Walter J., Parfrey L. W., Cooper P. J. & Finlay B. 2018. Associations between infant fungal and bacterial dysbiosis and childhood atopic wheeze in a nonindustrialized setting. *J. Allergy Clin. Immunol.*, 142:424-434.e10. doi: 10.1016/j.jaci.2017.08.041

Auchtung T. A., Fofanova T. Y., Stewart C. J., Nash A. K., Wong M. C., Gesell J. R., Auchtung J. M., Ajami N. J. & Petrosino J. F. 2018. Investigating Colonization of the Healthy Adult Gastrointestinal Tract by Fungi. *mSphere*, 3. doi: 10.1128/mSphere.00092-18

Audebert C., Even G., Cian A., Safadi D. El, Certad G., Delhaes L., Pereira B., Nourrisson C., Poirier P., Wawrzyniak I., Delbac F., Morelle C., Bastien P., Lachaud L., Bellanger

A.-P., Botterel F., Candolfi E., Desoubeaux G., Morio F., Pomares C., Rabodonirina M., Loywick A., Merlin S., Viscogliosi E. & Chabé M. 2016. Colonization with the enteric protozoa *Blastocystis* is associated with increased diversity of human gut bacterial microbiota. *Sci. Rep.*, 6:25255. doi: 10.1038/srep25255

Bär A.-K., Phukan N., Pinheiro J. & Simoes-Barbosa A. 2015. The Interplay of Host Microbiota and Parasitic Protozoans at Mucosal Interfaces: Implications for the Outcomes of Infections and Diseases. *PLoS Negl. Trop. Dis.*, 9:e0004176. doi: 10.1371/journal.pntd.0004176

Barash N. R., Maloney J. G., Singer S. M. & Dawson S. C. 2017. *Giardia* Alters Commensal Microbial Diversity throughout the Murine Gut. *Infect. Immun.*, 85. doi: 10.1128/IAI.00948-16

Barc M.-C., Charrin-Sarnel C., Rochet V., Bourlioux F., Sandré C., Boureau H., Doré J. & Collignon A. 2008. Molecular analysis of the digestive microbiota in a gnotobiotic mouse model during antibiotic treatment: Influence of Saccharomyces boulardii. *Anaerobe*, 14:229-33. doi: 10.1016/j.anaerobe.2008.04.003

Bartelt L. A. & Platts-Mills J. A. 2016. *Giardia*: a pathogen or commensal for children in high prevalence settings? *Curr. Opin. Infect. Dis.*, 29:502–507. doi: 10.1097/QCO.0000000000000293

Beghini F., Pasolli E., Truong T. D., Putignani L., Cacciò S. M. & Segata N. 2017. Large-scale comparative metagenomics of *Blastocystis*, a common member of the human gut microbiome. *ISME J.*, 11:2848-2863. doi: 10.1038/ismej.2017.139

Blaalid R., Kumar S., Nilsson R. H., Abarenkov K., Kirk P. M. & Kauserud H. 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. *Mol. Ecol. Resour.*, 13:218-24. doi: 10.1111/1755-0998.12065

Bouzid M., Hunter P. R., Chalmers R. M. & Tyler K. M. 2013. Cryptosporidium Pathogenicity and Virulence. *Clin. Microbiol. Rev.*, 26:115–134. doi: 10.1128/CMR.00076-12

Boyle R. J., Robins-Browne R. M. & Tang M. L. 2006. Probiotic use in clinical practice: what are the risks? *Am. J. Clin. Nutr.*, 83:1256-1264. doi: 10.1093/ajcn/83.6.1256

Bronstein J. L. 1994. Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol.*, 9:214-7. doi: 10.1016/0169-5347(94)90246-1

Bronstein J. L. 2001. The exploitation of mutualisms. *Ecol. Lett.*, 4:277-287. doi: 10.1046/j.1461-0248.2001.00218.x

Bruijnesteijn van Coppenraet L. E. S., Dullaert-de Boer M., Ruijs G. J. H. M., van der Reijden W. A., van der Zanden A. G. M., Weel J. F. L. & Schuurs T. A. 2015. Case–control comparison of bacterial and protozoan microorganisms associated with gastroenteritis: application of molecular detection. *Clin. Microbiol. Infect.*, 21:592.e9-592.e19. doi: 10.1016/j.cmi.2015.02.007

Chabé M., Lokmer A. & Ségurel L. 2017. Gut Protozoa: Friends or Foes of the Human Gut Microbiota? *Trends Parasitol.*, 33:925-934. doi: 10.1016/j.pt.2017.08.005

Chaucheyras-Durand F. & Durand H. 2010. Probiotics in animal nutrition and health. *Benef. Microbes*, 1:3-9. doi: 10.3920/BM2008.1002

Chen Y. E., Fischbach M. A. & Belkaid Y. 2018. Skin microbiota-host interactions. *Nature*, 553:427-436. doi: 10.1038/nature25177

Chudnovskiy A., Mortha A., Kana V., Kennard A., Ramirez J. D., Rahman A., Remark R., Mogno I., Ng R., Gnjatic S., Amir E.-A. D., Solovyov A., Greenbaum B., Clemente J., Faith J., Belkaid Y., Grigg M. E. & Merad M. 2016. Host-Protozoan Interactions Protect from Mucosal Infections through Activation of the Inflammasome. *Cell*, 167:444-456.e14. doi: 10.1016/j.cell.2016.08.076

Cortés A., Peachey L. E., Jenkins T. P., Scotti R. & Cantacessi C. 2019. Helminths and microbes within the vertebrate gut – not all studies are created equal. *Parasitology*, 146:1371–1378. doi: 10.1017/S003118201900088X

Costello E. K., Stagaman K., Dethlefsen L., Bohannan B. J. M. & Relman D. A. 2012. The application of ecological theory toward an understanding of the human microbiome. *Science*, 336:1255-62. doi: 10.1126/science.1224203

Coyte K. Z., Schluter J. & Foster K. R. 2015. The ecology of the microbiome: Networks, competition, and stability. *Science*, 350:663-6. doi: 10.1126/science.aad2602

Criseo G., Scordino F. & Romeo O. 2015. Current methods for identifying clinically important cryptic *Candida* species. *J. Microbiol. Methods*, 111:50–56. doi: 10.1016/j.mimet.2015.02.004

Cui L., Morris A. & Ghedin E. 2013. The human mycobiome in health and disease. *Genome Med.*, 5:63. doi: 10.1186/gm467

Cushman J. H. & Whitham T. G. 1989. Conditional Mutualism in a Membracid-Ant Association: Temporal, Age-Specific, and Density-Dependent Effects. *Ecology*, 70:1040-1047. doi: 10.2307/1941372

Czerucka D., Piche T. & Rampal P. 2007. Review article: yeast as probiotics -- Saccharomyces boulardii. *Aliment. Pharmacol. Ther.*, 26:767-78. doi: 10.1111/j.1365-2036.2007.03442.x

David L. A., Maurice C. F., Carmody R. N., Gootenberg D. B., Button J. E., Wolfe B. E., Ling A. V, Devlin A. S., Varma Y., Fischbach M. A., Biddinger S. B., Dutton R. J. & Turnbaugh P. J. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, 505:559-63. doi: 10.1038/nature12820

Dethlefsen L., McFall-Ngai M. & Relman D. A. 2007. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature*, 449:811-8. doi: 10.1038/nature06245

Dolinšek J., Goldschmidt F. & Johnson D. R. 2016. Synthetic microbial ecology and the dynamic interplay between microbial genotypes. *FEMS Microbiol. Rev.*, 40:961-979. doi: 10.1093/femsre/fuw024

Duvallet C., Gibbons S. M., Gurry T., Irizarry R. A. & Alm E. J. 2017. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nat. Commun.*, 8:1784. doi: 10.1038/s41467-017-01973-8

Eckburg P. B., Bik E. M., Bernstein C. N., Purdom E., Dethlefsen L., Sargent M., Gill S. R., Nelson K. E. & Relman D. A. 2005. Diversity of the human intestinal microbial flora. *Science*, 308:1635-8. doi: 10.1126/science.1110591

Edwards-Ingram L., Gitsham P., Burton N., Warhurst G., Clarke I., Hoyle D., Oliver S. G. & Stateva L. 2007. Genotypic and physiological characterization of Saccharomyces boulardii, the probiotic strain of Saccharomyces cerevisiae. *Appl. Environ. Microbiol.*, 73:2458-67. doi: 10.1128/AEM.02201-06

Eisen J. A. 2017. Microbiomania and 'overselling the microbiome.' https://phylogenomics.blogspot.com/p/blog-page.html. Accessed 5 Aug 2019.

Elzinga J., van der Oost J., de Vos W. M. & Smidt H. 2019. The Use of Defined Microbial Communities To Model Host-Microbe Interactions in the Human Gut. *Microbiol. Mol. Biol. Rev.*, 83. doi: 10.1128/MMBR.00054-18

Everard A., Lazarevic V., Gaïa N., Johansson M., Ståhlman M., Backhed F., Delzenne N. M., Schrenzel J., François P. & Cani P. D. 2014. Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity. *ISME J.*, 8:2116-30. doi: 10.1038/ismej.2014.45

Flaherty B. R., Talundzic E., Barratt J., Kines K. J., Olsen C., Lane M., Sheth M. & Bradbury

R. S. 2018. Restriction enzyme digestion of host DNA enhances universal detection of parasitic pathogens in blood via targeted amplicon deep sequencing. *Microbiome*, 6:164. doi: 10.1186/s40168-018-0540-2

Fleissner C. K., Huebel N., Abd El-Bary M. M., Loh G., Klaus S. & Blaut M. 2010. Absence of intestinal microbiota does not protect mice from diet-induced obesity. *Br. J. Nutr.*, 104:919-29. doi: 10.1017/S0007114510001303

Foster K. R., Schluter J., Coyte K. Z. & Rakoff-Nahoum S. 2017. The evolution of the host microbiome as an ecosystem on a leash. *Nature*, 548:43-51. doi: 10.1038/nature23292

Fujimura K. E., Sitarik A. R., Havstad S., Lin D. L., Levan S., Fadrosh D., Panzer A. R., LaMere B., Rackaityte E., Lukacs N. W., Wegienka G., Boushey H. A., Ownby D. R., Zoratti E. M., Levin A. M., Johnson C. C. & Lynch S. V. 2016. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat. Med.*, 22:1187-1191. doi: 10.1038/nm.4176

Gevers D., Kugathasan S., Knights D., Kostic A. D., Knight R. & Xavier R. J. 2017. A microbiome foundation for the study of Crohn's disease. *Cell Host Microbe*, 21:301-304. doi: 10.1016/j.chom.2017.02.012

Ghannoum M. 2016. Cooperative Evolutionary Strategy between the Bacteriome and Mycobiome. *MBio*, 7. doi: 10.1128/mBio.01951-16

Ghannoum M., Jurevic R. J., Mukherjee P. K., Cui F., Sikaroodi M., Naqvi A. & Gillevet P. M. 2010. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. *PLoS Pathog.*, 6:e1000713. doi: 10.1371/journal.ppat.1000713

Gillevet P. M., Sikaroodi M. & Torzilli A. P. 2009. Analyzing salt-marsh fungal diversity: comparing ARISA fingerprinting with clone sequencing and pyrosequencing. *Fungal Ecol.*, 2:160-167. doi: 10.1016/j.funeco.2009.04.001

Hallen-Adams H. E., Kachman S. D., Kim J., Legge R. M. & Martínez I. 2015. Fungi inhabiting the healthy human gastrointestinal tract: a diverse and dynamic community. *Fungal Ecol.*, 15:9-17. doi: 10.1016/j.funeco.2015.01.006

Hallen-Adams H. E. & Suhr M. J. 2017. Fungi in the healthy human gastrointestinal tract. *Virulence*, 8:352-358. doi: 10.1080/21505594.2016.1247140

Hamad I., Raoult D. & Bittar F. 2016. Repertory of eukaryotes (eukaryome) in the human gastrointestinal tract: taxonomy and detection methods. *Parasite Immunol.*, 38:12-36. doi: 10.1111/pim.12284

Hamad I., Sokhna C., Raoult D. & Bittar F. 2012. Molecular detection of eukaryotes in a single human stool sample from senegal. *PLoS One*, 7. doi: 10.1371/journal.pone.0040888

Hammer T., Janzen D. H., Hallwachs W., Jaffe S. P. & Fierer N. 2017. Caterpillars lack a resident gut microbiome. *Proc. Natl. Acad. Sci. U. S. A.*, 114:9641-9646. doi: 10.1073/pnas.1707186114

Hammer T., Sanders J. & Fierer N. 2018. Do all animals need microbes? http://fiererlab.org/2018/05/18/do-all-animals-need-microbes/. Accessed 5 Aug 2019.

Hanson B., Zhou Y., Bautista E. J., Urch B., Speck M., Silverman F., Muilenberg M., Phipatanakul W., Weinstock G., Sodergren E., Gold D. R. & Sordillo J. E. 2016. Characterization of the bacterial and fungal microbiome in indoor dust and outdoor air samples: a pilot study. *Environ. Sci. Process. Impacts*, 18:713-24. doi: 10.1039/c5em00639b

Harley I. T. W. & Karp C. L. 2012. Obesity and the gut microbiome: Striving for causality. *Mol. Metab.*, 1:21-31. doi: 10.1016/j.molmet.2012.07.002

Heintz-Buschart A., May P., Laczny C. C., Lebrun L. A., Bellora C., Krishna A., Wampach L., Schneider J. G., Hogan A., de Beaufort C. & Wilmes P. 2017. Integrated multi-omics

of the human gut microbiome in a case study of familial type 1 diabetes. *Nat. Microbiol.*, 2:16180. doi: 10.1038/nmicrobiol.2016.180

Heisel T., Montassier E., Johnson A., Al-Ghalith G., Lin Y.-W., Wei L.-N., Knights D. & Gale C. A. 2017. High-Fat Diet Changes Fungal Microbiomes and Interkingdom Relationships in the Murine Gut. *mSphere*, 2. doi: 10.1128/mSphere.00351-17

Hempel S., Newberry S. J., Maher A. R., Wang Z., Miles J. N. V, Shanman R., Johnsen B. & Shekelle P. G. 2012. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA*, 307:1959-69. doi: 10.1001/jama.2012.3507

Hillesland K. L. 2018. Evolution on the bright side of life: microorganisms and the evolution of mutualism. *Ann. N. Y. Acad. Sci.*, 1422:88-103. doi: 10.1111/nyas.13515

Hoarau G., Mukherjee P. K., Gower-Rousseau C., Hager C., Chandra J., Retuerto M. A., Neut C., Vermeire S., Clemente J., Colombel J. F., Fujioka H., Poulain D., Sendid B. & Ghannoum M. A. 2016. Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn's Disease. *MBio*, 7. doi: 10.1128/mBio.01250-16

Hoffmann C., Dollive S., Grunberg S., Chen J., Li H., Wu G. D., Lewis J. D. & Bushman F. D. 2013. Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PLoS One*, 8:e66019. doi: 10.1371/journal.pone.0066019

Hooks K. B., Konsman J. P. & O'Malley M. A. 2018. Microbiota-gut-brain research: A critical analysis. *Behav. Brain Sci.*, :1-40. doi: 10.1017/S0140525X18002133

Hooks K. B. & O'Malley M. A. 2017. Dysbiosis and its discontents. *MBio*, 8:e01492-17. doi: 10.1128/mBio.01492-17

Huffnagle G. B. & Noverr M. C. 2013. The emerging world of the fungal microbiome. *Trends Microbiol.*, 21:334-41. doi: 10.1016/j.tim.2013.04.002

Huseyin C. E., O'Toole P. W., Cotter P. D. & Scanlan P. D. 2017. Forgotten fungi—the gut mycobiome in human health and disease. *FEMS Microbiol. Rev.*, 41:479-511. doi: 10.1093/femsre/fuw047

Iliev I. D., Funari V. A., Taylor K. D., Nguyen Q., Reyes C. N., Strom S. P., Brown J., Becker C. A., Fleshner P. R., Dubinsky M., Rotter J. I., Wang H. L., McGovern D. P. B., Brown G. D. & Underhill D. M. 2012. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science*, 336:1314-7. doi: 10.1126/science.1221789

Iliev I. D. & Leonardi I. 2017. Fungal dysbiosis: immunity and interactions at mucosal barriers. *Nat. Rev. Immunol.*, 17:635-646. doi: 10.1038/nri.2017.55

Jiang H., Ling Z., Zhang Y., Mao H., Ma Z., Yin Y., Wang W., Tang W., Tan Z., Shi J., Li L. & Ruan B. 2015. Altered fecal microbiota composition in patients with major depressive disorder. *Brain. Behav. Immun.*, 48:186-94. doi: 10.1016/j.bbi.2015.03.016

Jiang T., Shao T.-Y., Ang W. X. G., Kinder J. M., Turner L. H., Pham G., Whitt J., Alenghat T. & Way S. S. 2017. Commensal Fungi Recapitulate the Protective Benefits of Intestinal Bacteria. *Cell Host Microbe*, 22:809-816.e4. doi: 10.1016/j.chom.2017.10.013

Kabir S., Rahman M. M., Rahman M. B., Rahman M. M. & Ahmed S. U. 2004. The Dynamics of Probiotics on Growth Performance and Immune Response in Broilers. *Int. J. Poult. Sci.*, 3:361-364. doi: 10.3923/ijps.2004.361.364

Kaneshiro E. S. & Dei-Cas E. 2009. Why the International Workshops on Opportunistic Protists? *Eukaryot. Cell*, 8:426-8. doi: 10.1128/EC.00299-08

Kaplan J. E., Jones J. L. & Dykewicz C. A. 2000. Protists as opportunistic pathogens: public health impact in the 1990s and beyond. *J. Eukaryot. Microbiol.*, 47:15-20.

Keeling P. J. & del Campo J. 2017. Marine Protists Are Not Just Big Bacteria. *Curr. Biol.*, 27:R541-R549. doi: 10.1016/j.cub.2017.03.075

Koenig J. E., Spor A., Scalfone N., Fricker A. D., Stombaugh J., Knight R., Angenent L. T. & Ley R. E. 2011. Succession of microbial consortia in the developing infant gut microbiome. *Proc. Natl. Acad. Sci. U. S. A.*, 108 Suppl:4578-85. doi: 10.1073/pnas.1000081107

Kostic A. D., Gevers D., Siljander H., Vatanen T., Hyötyläinen T., Hämäläinen A.-M., Peet A., Tillmann V., Pöhö P., Mattila I., Lähdesmäki H., Franzosa E. A., Vaarala O., de Goffau M., Harmsen H., Ilonen J., Virtanen S. M., Clish C. B., Orešič M., Huttenhower C., Knip M., DIABIMMUNE Study Group & Xavier R. J. 2015. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe*, 17:260-73. doi: 10.1016/j.chom.2015.01.001

Laforest-Lapointe I. & Arrieta M.-C. 2018. Microbial Eukaryotes: a Missing Link in Gut Microbiome Studies. *mSystems*, 3. doi: 10.1128/mSystems.00201-17

Lara-Flores M., Olvera-Novoa M. A., Guzmán-Méndez B. E. & López-Madrid W. 2003. Use of the bacteria Streptococcus faecium and Lactobacillus acidophilus, and the yeast Saccharomyces cerevisiae as growth promoters in Nile tilapia (Oreochromis niloticus). *Aquaculture*, 216:193-201. doi: 10.1016/S0044-8486(02)00277-6

LaTuga M. S., Ellis J. C., Cotton C. M., Goldberg R. N., Wynn J. L., Jackson R. B. & Seed P. C. 2011. Beyond bacteria: a study of the enteric microbial consortium in extremely low birth weight infants. *PLoS One*, 6:e27858. doi: 10.1371/journal.pone.0027858

Levy M., Thaiss C. A. & Elinav E. 2016. Metabolites: messengers between the microbiota and the immune system. *Genes Dev.*, 30:1589–1597. doi: 10.1101/gad.284091.116

Lewis J. D., Chen E. Z., Baldassano R. N., Otley A. R., Griffiths A. M., Lee D., Bittinger K., Bailey A., Friedman E. S., Hoffmann C., Albenberg L., Sinha R., Compher C., Gilroy E., Nessel L., Grant A., Chehoud C., Li H., Wu G. D. & Bushman F. D. 2015. Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn's disease. *Cell Host Microbe*, 18:489-500. doi: 10.1016/j.chom.2015.09.008

Ley R. E., Peterson D. A. & Gordon J. I. 2006. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, 124:837-48. doi: 10.1016/j.cell.2006.02.017

Li Q., Wang C., Tang C., He Q. & Li J. 2014. Lymphocyte depletion after alemtuzumab induction disrupts intestinal fungal microbiota in cynomolgus monkeys. *Transplantation*, 98:951-9. doi: 10.1097/TP.0000000000000373

Libertucci J. & Young V. B. 2019. The role of the microbiota in infectious diseases. *Nat. Microbiol.*, 4:35-45. doi: 10.1038/s41564-018-0278-4

Liguori G., Lamas B., Richard M. L., Brandi G., da Costa G., Hoffmann T. W., Di Simone M. P., Calabrese C., Poggioli G., Langella P., Campieri M. & Sokol H. 2016. Fungal Dysbiosis in Mucosa-associated Microbiota of Crohn's Disease Patients. *J. Crohns. Colitis*, 10:296-305. doi: 10.1093/ecco-jcc/jjv209

Lloyd-Price J., Abu-Ali G. & Huttenhower C. 2016. The healthy human microbiome. *Genome Med.*, 8:51. doi: 10.1186/s13073-016-0307-y

Lukeš J., Kuchta R., Scholz T. & Pomajbíková K. 2014. (Self-) infections with parasites: re-interpretations for the present. *Trends Parasitol.*, 30:377–385. doi: 10.1016/j.pt.2014.06.005

Lukeš J., Stensvold C. R., Jirků-Pomajbíková K. & Wegener Parfrey L. 2015. Are Human Intestinal Eukaryotes Beneficial or Commensals? *PLoS Pathog.*, 11:e1005039. doi: 10.1371/journal.ppat.1005039

Lynch K., Parke E. & O'Malley M. 2019. How causal are microbiomes? A comparison with the Helicobacter pylori explanation of ulcers. *Biol. Philos.*

Maizels R. M. & McSorley H. J. 2016. Regulation of the host immune system by helminth parasites. *J. Allergy Clin. Immunol.*, 138:666–675. doi: 10.1016/j.jaci.2016.07.007

Manor O., Levy R. & Borenstein E. 2014. Mapping the inner workings of the microbiome: genomic- and metagenomic-based study of metabolism and metabolic interactions in the human microbiome. *Cell Metab.*, 20:742-752. doi: 10.1016/j.cmet.2014.07.021

Mar Rodríguez M., Pérez D., Javier Chaves F., Esteve E., Marin-Garcia P., Xifra G., Vendrell J., Jové M., Pamplona R., Ricart W., Portero-Otin M., Chacón M. R. & Fernández Real J. M. 2015. Obesity changes the human gut mycobiome. *Sci. Rep.*, 5:14600. doi: 10.1038/srep14600

Marchesi J. R., Adams D. H., Fava F., Hermes G. D. A., Hirschfield G. M., Hold G., Quraishi M. N., Kinross J., Smidt H., Tuohy K. M., Thomas L. V, Zoetendal E. G. & Hart A. 2016. The gut microbiota and host health: a new clinical frontier. *Gut*, 65:330-9. doi: 10.1136/gutjnl-2015-309990

McFarland L. V. 2010. Systematic review and meta-analysis of Saccharomyces boulardii in adult patients. *World J. Gastroenterol.*, 16:2202-22.

McKay D. M. 2010. The immune response to and immunomodulation by *Hymenolepis diminuta*. *Parasitology*, 137:385–94. doi: 10.1017/S0031182009990886

McKay D. M. 2015. Not all parasites are protective. *Parasite Immunol.*, 37:324–332. doi: 10.1111/pim.12160

Minot S., Sinha R., Chen J., Li H., Keilbaugh S. A., Wu G. D., Lewis J. D. & Bushman F. D. 2011. The human gut virome: inter-individual variation and dynamic response to diet. *Genome Res.*, 21:1616-25. doi: 10.1101/gr.122705.111

Moissl-Eichinger C., Pausan M., Taffner J., Berg G., Bang C. & Schmitz R. A. 2018. Archaea Are Interactive Components of Complex Microbiomes. *Trends Microbiol.*, 26:70-85. doi: 10.1016/j.tim.2017.07.004

Morton E. R., Lynch J., Froment A., Lafosse S., Heyer E., Przeworski M., Blekhman R. & Ségurel L. 2015. Variation in Rural African Gut Microbiota Is Strongly Correlated with Colonization by Entamoeba and Subsistence. *PLoS Genet.*, 11:e1005658. doi: 10.1371/journal.pgen.1005658

Munroe R. 2015. Gut Fauna. https://xkcd.com/1471/. Accessed 5 Aug 2019

Mushegian A. A. & Ebert D. 2016. Rethinking "mutualism" in diverse host-symbiont communities. *Bioessays*, 38:100-8. doi: 10.1002/bies.201500074

Naglik J. R., Fidel P. L. & Odds F. C. 2008. Animal models of mucosal *Candida* infection. *FEMS Microbiol. Lett.*, 283:129-39. doi: 10.1111/j.1574-6968.2008.01160.x

Nam Y.-D. Do, Chang H.-W. W., Kim K.-H. H., Roh S. W., Kim M.-S. S., Jung M.-J. J., Lee S.-W. W., Kim J.-Y. Y., Yoon J.-H. H. & Bae J.-W. W. 2008. Bacterial, archaeal, and eukaryal diversity in the intestines of Korean people. *J. Microbiol.*, 46:491-501. doi: 10.1007/s12275-008-0199-7

Nash A. K., Auchtung T. A., Wong M. C., Smith D. P., Gesell J. R., Ross M. C., Stewart C. J., Metcalf G. A., Muzny D. M., Gibbs R. A., Ajami N. J. & Petrosino J. F. 2017. The gut mycobiome of the Human Microbiome Project healthy cohort. *Microbiome*, 5:153. doi: 10.1186/s40168-017-0373-4

Nguyen T. L. A., Vieira-Silva S., Liston A. & Raes J. 2015. How informative is the mouse for human gut microbiota research? *Dis. Model. Mech.*, 8:1–16. doi: 10.1242/dmm.017400

Nieves-Ramírez M. E., Partida-Rodríguez O., Laforest-Lapointe I., Reynolds L. A., Brown E. M., Valdez-Salazar A., Morán-Silva P., Rojas-Velázquez L., Morien E., Parfrey L. W., Jin M., Walter J., Torres J., Arrieta M. C., Ximénez-García C. & Finlay B. B. 2018. Asymptomatic Intestinal Colonization with Protist *Blastocystis* Is Strongly Associated

with Distinct Microbiome Ecological Patterns. *mSystems*, 3. doi: 10.1128/mSystems.00007-18

Nilsson R. H., Anslan S., Bahram M., Wurzbacher C., Baldrian P. & Tedersoo L. 2019. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nat. Rev. Microbiol.*, 17:95-109. doi: 10.1038/s41579-018-0116-y

Norman J. M., Handley S. A. & Virgin H. W. 2014. Kingdom-agnostic metagenomics and the importance of complete characterization of enteric microbial communities. *Gastroenterology*, 146:1459-69. doi: 10.1053/j.gastro.2014.02.001

O'Malley M. A. & Skillings D. J. 2018. Methodological Strategies in Microbiome Research and their Explanatory Implications. *Perspect. Sci.*, 26:239-265. doi: 10.1162/POSC_a_00274

Olesen S. W. & Alm E. J. 2016. Dysbiosis is not an answer. *Nat. Microbiol.*, 1:16228. doi: 10.1038/nmicrobiol.2016.228

Olle B. 2013. Medicines from microbiota. *Nat. Biotechnol.*, 31:309-15. doi: 10.1038/nbt.2548

Osman M., El Safadi D., Cian A., Benamrouz S., Nourrisson C., Poirier P., Pereira B., Razakandrainibe R., Pinon A., Lambert C., Wawrzyniak I., Dabboussi F., Delbac F., Favennec L., Hamze M., Viscogliosi E. & Certad G. 2016. Prevalence and Risk Factors for Intestinal Protozoan Infections with *Cryptosporidium*, *Giardia*, *Blastocystis* and *Dientamoeba* among Schoolchildren in Tripoli, Lebanon.Picado A. (ed.). *PLoS Negl. Trop. Dis.*, 10:e0004496. doi: 10.1371/journal.pntd.0004496

Ott S. J., Kühbacher T., Musfeldt M., Rosenstiel P., Hellmig S., Rehman A., Drews O., Weichert W., Timmis K. N. & Schreiber S. 2008. Fungi and inflammatory bowel diseases: Alterations of composition and diversity. *Scand. J. Gastroenterol.*, 43:831-41. doi: 10.1080/00365520801935434

Pandey P. K., Siddharth J., Verma P., Bavdekar A., Patole M. S. & Shouche Y. S. 2012. Molecular typing of fecal eukaryotic microbiota of human infants and their respective mothers. *J. Biosci.*, 37:221-6. doi: 10.1007/s12038-012-9197-3

Parfrey L. W., Walters W. A., Lauber C. L., Clemente J. C., Berg-Lyons D., Teiling C., Kodira C., Mohiuddin M., Brunelle J., Driscoll M., Fierer N., Gilbert J. A. & Knight R. 2014. Communities of microbial eukaryotes in the mammalian gut within the context of environmental eukaryotic diversity. *Front. Microbiol.*, 5:298. doi: 10.3389/fmicb.2014.00298

Parker W. & Ollerton J. 2013. Evolutionary biology and anthropology suggest biome reconstitution as a necessary approach toward dealing with immune disorders. *Evol. Med. Public Heal.*, 2013:89–103. doi: 10.1093/emph/eot008

Paulino L. C., Tseng C.-H., Strober B. E. & Blaser M. J. 2006. Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. *J. Clin. Microbiol.*, 44:2933-41. doi: 10.1128/JCM.00785-06

Phillips A. W. & Balish E. 1966. Growth and invasiveness of *Candida albicans* in the germ-free and conventional mouse after oral challenge. *Appl. Microbiol.*, 14:737-41.

Postler T. S. & Ghosh S. 2017. Understanding the Holobiont: How Microbial Metabolites Affect Human Health and Shape the Immune System. *Cell Metab.*, 26:110–130. doi: 10.1016/j.cmet.2017.05.008

Qin J., Li R., Raes J., Arumugam M., Burgdorf K. S., Manichanh C., Nielsen T., Pons N., Levenez F., Yamada T., Mende D. R., Li J., Xu J., Li Shaochuan, Li D., Cao J., Wang B., Liang H., Zheng H., Xie Y., Tap J., Lepage P., Bertalan M., Batto J.-M., Hansen T., Le Paslier D., Linneberg A., Nielsen H. B., Pelletier E., Renault P., Sicheritz-Ponten T., Turner K., Zhu H., Yu C., Li Shengting, Jian M., Zhou Y., Li Y., Zhang X., Li

Songgang, Qin N., Yang H., Wang Jian, Brunak S., Doré J., Guarner F., Kristiansen K., Pedersen O., Parkhill J., Weissenbach J., Bork P., Ehrlich S. D. & Wang Jun. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, 464:59–65. doi: 10.1038/nature08821

Rapin A. & Harris N. L. 2018. Helminth–Bacterial Interactions: Cause and Consequence. *Trends Immunol.*, 39:724–733. doi: 10.1016/j.it.2018.06.002

Rauch M. & Lynch S. V. 2012. The potential for probiotic manipulation of the gastrointestinal microbiome. *Curr. Opin. Biotechnol.*, 23:192-201. doi: 10.1016/j.copbio.2011.11.004

Relman D. A. 2015. The human microbiome and the future practice of medicine. *JAMA*, 314:1127-8. doi: 10.1001/jama.2015.10700

Reynolds L. A., Finlay B. B. & Maizels R. M. 2015. Cohabitation in the Intestine: Interactions among Helminth Parasites, Bacterial Microbiota, and Host Immunity. *J. Immunol.*, 195:4059-66. doi: 10.4049/jimmunol.1501432

Richard M. L., Lamas B., Liguori G., Hoffmann T. W. & Sokol H. 2015. Gut fungal microbiota: the Yin and Yang of inflammatory bowel disease. *Inflamm. Bowel Dis.*, 21:656-65. doi: 10.1097/MIB.0000000000000261

Richardson M. D. 1991. Opportunistic and pathogenic fungi. *J. Antimicrob. Chemother.*, 28 Suppl A:1-11.

Rocchi S., Valot B., Reboux G. & Millon L. 2017. DNA metabarcoding to assess indoor fungal communities: Electrostatic dust collectors and Illumina sequencing. *J. Microbiol. Methods*, 139:107-112. doi: 10.1016/j.mimet.2017.05.014

Ross B. D., Hayes B., Radey M. C., Lee X., Josek T., Bjork J., Neitzel D., Paskewitz S., Chou S. & Mougous J. D. 2018. Ixodes scapularis does not harbor a stable midgut microbiome. *ISME J.*, 12:2596-2607. doi: 10.1038/s41396-018-0161-6

Rowan-Nash A. D., Korry B. J., Mylonakis E. & Belenky P. 2019. Cross-Domain and Viral Interactions in the Microbiome. *Microbiol. Mol. Biol. Rev.*, 83. doi: 10.1128/MMBR.00044-18

De Roy K., Marzorati M., Van den Abbeele P., Van de Wiele T. & Boon N. 2014. Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities. *Environ. Microbiol.*, 16:1472-81. doi: 10.1111/1462-2920.12343

Scales B. S., Dickson R. P., LiPuma J. J. & Huffnagle G. B. 2014. Microbiology, genomics, and clinical significance of the Pseudomonas fluorescens species complex, an unappreciated colonizer of humans. *Clin. Microbiol. Rev.*, 27:927-48. doi: 10.1128/CMR.00044-14

Scanlan P. D. 2012. *Blastocystis*: past pitfalls and future perspectives. *Trends Parasitol.*, 28:327-34. doi: 10.1016/j.pt.2012.05.001

Scanlan P. D., Hill C. J., Ross R. P., Ryan C. A., Stanton C. & Cotter P. D. 2018. The intestinal protist *Blastocystis* is not a common member of the healthy infant gut microbiota in a Westernized country (Ireland). *Parasitology*, 145:1274–1278. doi: 10.1017/S0031182018000033

Scanlan P. D. & Marchesi J. R. 2008. Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces. *ISME J.*, 2:1183-93. doi: 10.1038/ismej.2008.76

Schoch C. L., Seifert K. A., Huhndorf S., Robert V., Spouge J. L., Levesque C. A., Chen W., Fungal Barcoding Consortium & Fungal Barcoding Consortium Author List. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. U. S. A.*, 109:6241-6. doi: 10.1073/pnas.1117018109

Severance E. G., Gressitt K. L., Stallings C. R., Katsafanas E., Schweinfurth L. A., Savage C. L. G., Adamos M. B., Sweeney K. M., Origoni A. E., Khushalani S., Dickerson F. B. & Yolken R. H. 2017. Probiotic normalization of *Candida albicans* in schizophrenia: A randomized, placebo-controlled, longitudinal pilot study. *Brain. Behav. Immun.*, 62:41-45. doi: 10.1016/j.bbi.2016.11.019

Shade A. 2017. Diversity is the question, not the answer. *ISME J.*, 11:1-6. doi: 10.1038/ismej.2016.118

Shi Y., Pan C., Wang K., Chen X., Wu X., Chen C.-T. A. & Wu B. 2017. Synthetic multispecies microbial communities reveals shifts in secondary metabolism and facilitates cryptic natural product discovery. *Environ. Microbiol.*, 19:3606-3618. doi: 10.1111/1462-2920.13858

Shirtliff M. E., Peters B. M. & Jabra-Rizk M. A. 2009. Cross-kingdom interactions: *Candida albicans* and bacteria. *FEMS Microbiol. Lett.*, 299:1-8. doi: 10.1111/j.1574-6968.2009.01668.x

Simon O., Jadamus A. & Vahjen W. 2001. Probiotic feed additives - effectiveness and expected modes of action. *J. Anim. Feed Sci.*, 10:51-67. doi: 10.22358/jafs/70012/2001

Sokol H., Leducq V., Aschard H., Pham H.-P., Jegou S., Landman C., Cohen D., Liguori G., Bourrier A., Nion-Larmurier I., Cosnes J., Seksik P., Langella P., Skurnik D., Richard M. L. & Beaugerie L. 2017. Fungal microbiota dysbiosis in IBD. *Gut*, 66:1039-1048. doi: 10.1136/gutjnl-2015-310746

Sonnenburg E. D., Smits S. A., Tikhonov M., Higginbottom S. K., Wingreen N. S. & Sonnenburg J. L. 2016. Diet-induced extinctions in the gut microbiota compound over generations. *Nature*, 529:212-5. doi: 10.1038/nature16504

Sovran B., Planchais J., Jegou S., Straube M., Lamas B., Natividad J. M., Agus A., Dupraz L., Glodt J., Da Costa G., Michel M.-L., Langella P., Richard M. L. & Sokol H. 2018. Enterobacteriaceae are essential for the modulation of colitis severity by fungi. *Microbiome*, 6:152. doi: 10.1186/s40168-018-0538-9

Stappenbeck T. S. & Virgin H. W. 2016. Accounting for reciprocal host-microbiome interactions in experimental science. *Nature*, 534:191-9. doi: 10.1038/nature18285

Stein R. R., Bucci V., Toussaint N. C., Buffie C. G., Rätsch G., Pamer E. G., Sander C. & Xavier J. B. 2013. Ecological modeling from time-series inference: insight into dynamics and stability of intestinal microbiota. *PLoS Comput. Biol.*, 9:31-36. doi: 10.1371/journal.pcbi.1003388

Stensvold C. & van der Giezen M. 2018. Associations between Gut Microbiota and Common Luminal Intestinal Parasites. *Trends Parasitol.*, 34:369-377. doi: 10.1016/j.pt.2018.02.004

Stensvold C., Traub R. J., von Samson-Himmelstjerna G., Jespersgaard C., Nielsen H. V & Thompson R. C. A. 2007. *Blastocystis*: subtyping isolates using pyrosequencing technology. *Exp. Parasitol.*, 116:111-9. doi: 10.1016/j.exppara.2006.12.002

Suez J., Zmora N., Segal E. & Elinav E. 2019. The pros, cons, and many unknowns of probiotics. *Nat. Med.*, 25:716-729. doi: 10.1038/s41591-019-0439-x

Suhr M. J., Banjara N. & Hallen-Adams H. E. 2016. Sequence-based methods for detecting and evaluating the human gut mycobiome. *Lett. Appl. Microbiol.*, 62:209-15. doi: 10.1111/lam.12539

Suhr M. J. & Hallen-Adams H. E. 2016. The human gut mycobiome: pitfalls and potentials--a mycologist's perspective. *Mycologia*, 107:1057-73. doi: 10.3852/15-147

Sze M. A. & Schloss P. D. 2016. Looking for a signal in the noise: revisiting obesity and the microbiome. *MBio*, 7:e01018-16. doi: 10.1128/mBio.01018-16

Thongsripong P., Chandler J. A., Green A. B., Kittayapong P., Wilcox B. A., Kapan D. D. &

Bennett S. N. 2018. Mosquito vector-associated microbiota: Metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. *Ecol. Evol.*, 8:1352-1368. doi: 10.1002/ece3.3676

Tso G. H. W., Reales-Calderon J. A., Tan A. S. M., Sem X., Le G. T. T., Tan T. G., Lai G. C., Srinivasan K. G., Yurieva M., Liao W., Poidinger M., Zolezzi F., Rancati G. & Pavelka N. 2018. Experimental evolution of a fungal pathogen into a gut symbiont. *Science*, 362:589-595. doi: 10.1126/science.aat0537

Turnbaugh P. J., Ley R. E., Mahowald M. A., Magrini V., Mardis E. R. & Gordon J. I. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444:1027-31. doi: 10.1038/nature05414

Vanhee L. M. E., Goemé F., Nelis H. J. & Coenye T. 2010. Quality control of fifteen probiotic products containing Saccharomyces boulardii. *J. Appl. Microbiol.*, 109:1745-52. doi: 10.1111/j.1365-2672.2010.04805.x

Vega N. M. & Gore J. 2018. Simple organizing principles in microbial communities. *Curr. Opin. Microbiol.*, 45:195-202. doi: 10.1016/j.mib.2018.11.007

Venturelli O. S., Carr A. C., Fisher G., Hsu R. H., Lau R., Bowen B. P., Hromada S., Northen T. & Arkin A. P. 2018. Deciphering microbial interactions in synthetic human gut microbiome communities. *Mol. Syst. Biol.*, 14:e8157. doi: 10.15252/msb.20178157

Viaud S., Daillère R., Boneca I. G., Lepage P., Pittet M. J., Ghiringhelli F., Trinchieri G., Goldszmid R. & Zitvogel L. 2014. Harnessing the intestinal microbiome for optimal therapeutic immunomodulation. *Cancer Res.*, 74:4217-21. doi: 10.1158/0008-5472.CAN-14-0987

Walsh C. J., Guinane C. M., O'Toole P. W. & Cotter P. D. 2014. Beneficial modulation of the gut microbiota. *FEBS Lett.*, 588:4120-30. doi: 10.1016/j.febslet.2014.03.035

Walters W. A., Xu Z. & Knight R. 2014. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett.*, 588:4223-33. doi: 10.1016/j.febslet.2014.09.039

Wampach L., Heintz-Buschart A., Hogan A., Muller E. E. L., Narayanasamy S., Laczny C. C., Hugerth L. W., Bindl L., Bottu J., Andersson A. F., de Beaufort C. & Wilmes P. 2017. Colonization and Succession within the Human Gut Microbiome by Archaea, Bacteria, and Microeukaryotes during the First Year of Life. *Front. Microbiol.*, 8:738. doi: 10.3389/fmicb.2017.00738

Ward T. L., Dominguez-Bello M. G., Heisel T., Al-Ghalith G., Knights D. & Gale C. A. 2018. Development of the Human Mycobiome over the First Month of Life and across Body Sites. *mSystems*, 3. doi: 10.1128/mSystems.00140-17

Watanabe K., Gilchrist C. A., Uddin M. J., Burgess S. L., Abhyankar M. M., Moonah S. N., Noor Z., Donowitz J. R., Schneider B. N., Arju T., Ahmed E., Kabir M., Alam M., Haque R., Pramoonjago P., Mehrad B. & Petri W. A. 2017. Microbiome-mediated neutrophil recruitment via CXCR2 and protection from amebic colitis. *PLoS Pathog.*, 13:e1006513. doi: 10.1371/journal.ppat.1006513

Westwater C., Balish E., Warner T. F., Nicholas P. J., Paulling E. E. & Schofield D. A. 2007. Susceptibility of gnotobiotic transgenic mice (Tgepsilon26) with combined deficiencies in natural killer cells and T cells to wild-type and hyphal signalling-defective mutants of *Candida albicans*. *J. Med. Microbiol.*, 56:1138-44. doi: 10.1099/jmm.0.47110-0

Wheeler M. L., Limon J. J., Bar A. S., Leal C. A., Gargus M., Tang J., Brown J., Funari V. A., Wang H. L., Crother T. R., Arditi M., Underhill D. M. & Iliev I. D. 2016. Immunological Consequences of Intestinal Fungal Dysbiosis. *Cell Host Microbe*, 19:865-73. doi: 10.1016/j.chom.2016.05.003

Yatsunenko T., Rey F. E., Manary M. J., Trehan I., Dominguez-Bello M. G., Contreras M., Magris M., Hidalgo G., Baldassano R. N., Anokhin A. P., Heath A. C., Warner B.,

Reeder J., Kuczynski J., Caporaso J. G., Lozupone C. A., Lauber C., Clemente J. C., Knights D., Knight R. & Gordon J. I. 2012. Human gut microbiome viewed across age and geography. *Nature*, 486:222-7. doi: 10.1038/nature11053

Zmora N., Zilberman-Schapira G., Suez J., Mor U., Dori-Bachash M., Bashiardes S., Kotler E., Zur M., Regev-Lehavi D., Brik R. B.-Z., Federici S., Cohen Y., Linevsky R., Rothschild D., Moor A. E., Ben-Moshe S., Harmelin A., Itzkovitz S., Maharshak N., Shibolet O., Shapiro H., Pevsner-Fischer M., Sharon I., Halpern Z., Segal E. & Elinav E. 2018. Personalized gut mucosal colonization resistance to empiric probiotics Is associated with unique host and microbiome features. *Cell*, 174:1388-1405.e21. doi: 10.1016/j.cell.2018.08.041

Zoetendal E. G., Ben-Amor K., Akkermans A. D., Abee T. & de Vos W. M. 2001. DNA isolation protocols affect the detection limit of PCR approaches of bacteria in samples from the human gastrointestinal tract. *Syst. Appl. Microbiol.*, 24:405-10. doi: 10.1078/0723-2020-00060

Zuo T., Wong S. H., Cheung C. P., Lam K., Lui R., Cheung K., Zhang F., Tang W., Ching J. Y. L., Wu J. C. Y., Chan P. K. S., Sung J. J. Y., Yu J., Chan F. K. L. & Ng S. C. 2018. Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in Clostridium difficile infection. *Nat. Commun.*, 9:3663. doi: 10.1038/s41467-018-06103-6

SUPPORTING INFORMATION

File S1. Supporting methods (see below)
Table S1. The list of 578 data-generating papers analysed in the study (separate file, available on request)

Literature and patent searches

International microbiome patents published between 2002 and 2018 were identified via an advanced search of the European Patent Office on 25/01/2019. We searched the 'Worldwide EN – collection of patents published in English' with the phrase "microbiome OR microbiota OR "gut flora" OR "gut microflora"". The yearly numbers of patents are shown in Fig. 1. To compare patent quantities to the quantity of microbiome literature, we searched PubMed with the same phrase and used the "Results by year" feature to extract data.

To identify literature targeting eukaryotic microbiomes (Fig. 2), we searched PubMed on 17/01/2019 with the phrase "28S OR 18S OR ITS1 OR ITS2 AND microbiome" " and a more general expression "Mycobiome OR mycobiota OR ("fungal microbiota") OR ("fungal microbiome") OR "eukaryotic microbiome" OR "eukaryotic microbiota" OR ("microeukaryotes AND sequencing")". In total we found 1091 unique journal articles from which we manually excluded 402. Reasons for exclusion included non-English publications, not full texts (e.g., short commentaries, editorials or errata), not focused on the eukaryotic microbiome (e.g., only mentioning it), and using exclusively culture methods rather than whole-community microbiome profiling techniques. Amongst the remaining articles we identified 111 (16%) reviews and 578 (84%) data-generating eukaryotic microbiome papers. Our focus was the papers generating new data rather than those reviewing other papers.

We assigned all data-generating papers to one of the following categories (in alphabetical order):

- Aquatic (65: e.g., ocean, sea sediments, hypersaline lagoons)
- Atmosphere (3)

- Biotechnology (62: e.g., cheese, wine production, fruit storage)
- Built environments (43: e.g., wastewater treatment sites, mines, buildings)
- Human (140: e.g., gut, skin, lungs)
- Other animals (90: e.g., mice, rumen, insects)
- Plants (74: e.g., endophytes, mycorrhiza)
- Soil (51: e.g. agricultural soil, biochar)
- Technical (37: experimental and computational methods for metagenomic sequencing analysis)
- Terrestrial (13: e.g., caves, rocks, volcanoes).

Assignment into these categories was based on reading the abstracts and, in some cases, the full paper. There is a continuum between the categories of 'terrestrial', 'soil', and 'plants'. Clearly soil is just one type of terrestrial environment, but it is specific and common enough to warrant its own theme. Plant root studies were categorized as belonging half to soil and half to plant microbiomes.

In addition, we manually searched the full text of all articles (689 data-generating and review papers) for 'dysbiosis', 'dysbiotic' or '(im)balanced microbiome' (8% of papers), and these findings are discussed in the text. The full list of 578 papers with their bibliometric data and assignment into categories is available in the Table S1 1.