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REVIEW ARTICLE

Intestinal microbiome landscaping: insight in community assemblage and implications for microbial modulation strategies

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One sentence summary: A meta-analysis-based review discusses the key features of human intestinal microbiome, i.e. the compositional and functional 'core', community types, and the existence of alternative stable states and how these concepts can be used to improve host health.
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ABSTRACT

High individuality, large complexity and limited understanding of the mechanisms underlying human intestinal microbiome function remain the major challenges for designing beneficial modulation strategies. Exemplified by the analysis of intestinal bacteria in a thousand Western adults, we discuss key concepts of the human intestinal microbiome landscape, i.e. the compositional and functional 'core', the presence of community types and the existence of alternative stable states. Genomic investigation of core taxa revealed functional redundancy, which is expected to stabilize the ecosystem, as well as taxa with specialized functions that have the potential to shape the microbiome landscape. The contrast between *Prevotella*- and *Bacteroides*-dominated systems has been well described. However, less known is the effect of not so abundant bacteria, for example, *Dialister* spp. that have been proposed to exhibit distinct bistable dynamics. Studies employing time-series analysis have highlighted the dynamical variation in the microbiome landscape with and without the effect of defined perturbations, such as the use of antibiotics or dietary changes. We incorporate ecosystem-level observations of the human intestinal microbiota and its keystone species to suggest avenues for designing microbiome modulation strategies to improve host health.

Keywords: alternative stable states; bistability; core microbiota; early warning signals; landscape model; tipping elements

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INTRODUCTION

Microbial cells may outnumber our own cells in the body, presenting an example of nature's intricate evolutionary scheme of host–microbe symbiosis (Frank and Pace 2008; Ley *et al.* 2008; Sender, Fuchs and Milo 2016). This unique setup of human–microbe interactions and its resilience is of paramount importance for health. Starting from birth, various factors shape the composition and function of the human intestinal tract microbiome, defined as the entire habitat including all microbes, their genomes and surrounding environment (see Table 1) (Sekirov *et al.* 2010; Ottman *et al.* 2012).

The human intestinal tract microbiota consists mainly of Bacteria, but also includes Archaea, microeukaryotes and many viruses, mainly bacteriophages. The dominant bacterial phyla are *Bacteroidetes* and *Firmicutes*, while *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* constitute minor phyla (Li *et al.* 2014; Falony *et al.* 2016). Archaeal representatives consist largely of methanogens, among which *Methanobrevibacter* and *Methanosphaera* are the most prevalent genera (Dridi *et al.* 2009). The microeukaryotes commonly observed in the human intestinal tract include *Blastocystis* spp. and fungal genera, such as *Candida* spp., *Gloetenia/Paecilomyces* and *Galactomyces*. Finally, various reports have detailed the presence of bacteriophages in the human intestinal tract (Minot *et al.* 2013; Waller *et al.* 2014). A recent deep genomic analysis of virus-like particles present in human fecal samples revealed that most bacteriophages belong to the *Caudovirales* order (*Podoviridae*, *Siphiridae* and *Myoviridae*), some to the *Microviridae* and no eukaryotic viral sequences were detected (Manrique *et al.* 2016). The latter study revealed 64 healthy subjects to harbor a total of 44 bacteriophage groups that included a set of core bacteriophages shared among over half of the subjects, a common set that was present in 20%–50% of the subjects, and a set of bacteriophages that are either rarely shared or unique to an individual. While there are indications that bacteriophages may be involved in controlling the intestinal microbiota in health and disease, additional deep studies are needed to assess how these phages are involved in the host–microbe interactions (Reyes *et al.* 2010, 2015; Manrique *et al.* 2016).

Interactions between the host, the prokaryotic and eukaryotic microbes and their viruses take place at various levels. While there are marked differences in microbial composition and function along the entire length of the intestinal tract, most research has focused on the fecal microbiota as fecal sampling is a cost-efficient and non-invasive method. However, the compositional and functional attributes of the microbiota vary along the intestinal tract—most notably, the small intestine harbors a unique microbial ecosystem that has been characterized extensively (Zoetendal *et al.* 2012; Zoetendal and de Vos 2014). Moreover, the fecal microbiota may differ from that in the colonic mucosa but systematic studies are lacking because of the invasiveness of the sampling (Zoetendal *et al.* 2002; Donaldson, Lee and Mazmanian 2016). As bacteria are the dominant group in this ecosystem, they have received most attention in the recent studies (Lozupone *et al.* 2012; Li *et al.* 2014; Falony *et al.* 2016). After birth, the infant intestinal tract becomes colonized by bacteria largely gained from the mother (Favier, de Vos and Akkermans 2003; Koren *et al.* 2012). The programmed outgrowth, manifested as succession, and the composition of the adult intestinal microbiota depend on a variety of deterministic host factors, including age, diet, genetic-make-up, immune and health status, and gender and geographical location (Biagi *et al.* 2010; De Filippo *et al.* 2010; Claesson *et al.* 2012; Yatsunenko *et al.* 2012). Furthermore, stochastic factors such as colonization order or antibiotic exposure can also affect microbial colonization (Lederberg and McCray 2001; Harris *et al.* 2015; Korpela *et al.* 2016). Overall, this process leads to the development of a dynamic and complex web of interactions between microorganisms, the host and the environment (Costello *et al.* 2012; Ottman *et al.* 2012). Knowledge of the deterministic and stochastic factors affecting the host–microbiota homeostasis could help in defining the factors responsible for evolutionary selection and establishment of the symbiosis between human and microbes.

Bacteria form the dominant domain in the large intestinal microbiota and hence we focus here on the accumulated knowledge of this group. For convenience, the definition of technical terminologies used in this review is given in Table 1. We discuss the concepts of the bacterial compositional and functional core, community types and alternative stable states of the microbial ecosystem. We will further discuss how improved understanding of these features could be instrumental in microbial diagnostics, predicting changes or homeostasis and determining the efficacy of intervention strategies for microbiome modulation.

THE COMMON CORE MICROBIOME IN THE HUMAN LARGE INTESTINE

A major goal in human microbiome studies is to identify and characterize the bacterial taxa and functions that are shared by the majority of individuals. Viewing the bacterial community from a taxonomic perspective reveals an increasing level of complexity toward the lower taxonomic ranks from phylum level to species level. Moreover, the intestinal bacteria interact with each other and the host at various levels from single cells to populations of specific bacteria to the entire community. The complexity of such multi-scale interactions presents a major challenge in understanding community functioning and its impact on host health (Greenblum, Turnbaugh and Borenstein 2012; Greenblum *et al.* 2013). One way to approach this challenge is to focus on the most abundant bacterial species and specifically the prevalent ones that are shared by many individuals.

Coevolution of host and microbiome can be explained by ecological selection processes (Pianka 1970; Ley, Peterson and Gordon 2006). Host factors such as genetics, immune regulation and age represent top–down selection that plays a role in shaping the microbial community. In contrast, bottom–up selection results in the growth of microbes that have specialized functions, such as those associated with degradation of specific dietary components. Another concept is the r/K selection where organisms that have adapted to maximize the rate of growth are so-called r-strategists, whereas those adapted to compete and survive when resources are limited are known as K-strategists (Pianka 1970). However, applying these idealized concepts to the highly complex, dense and dynamical intestinal ecosystem is challenging (Freilich *et al.* 2010). The large intestine is known to have spatial heterogeneity in terms of bacterial species which occupy microhabitats (Donaldson, Lee and Mazmanian 2016). For example, the outer mucus layer is densely populated compared to the inner mucus layer (Johansson *et al.* 2008). The presence of microhabitats with variable population densities suggests that, rather than being present at one of the two endpoints, the bacterial community should be viewed along the r-K continuum that is governed by top–down and bottom–up selection processes.

The aforementioned factors result in the selection of bacteria belonging to specific phylogenetic clusters with adaptive and functional features characteristic for the ecosystem. This

Table 1. Terms and definitions used in this review.

Terminologies	Definitions	Reference
Active functional core	The common functions that are translated and translated actively across human population and have an effect on the whole ecosystem.	Kolmeder *et al.* (2012)
Alternative stable states	Resilient community states that are stable and resist change over ecologically relevant timescales.	Fukami and Nakajima (2011)
Alternative transient states	The community states that are not necessarily stable and vary in structure and/or function.	Fukami and Nakajima (2011)
Bacterial phylogenetic core	The assemblage of phylogenetically related bacterial taxa in the human intestinal microbiome.	Rajilić-Stojanović *et al.* (2009); Tap *et al.* (2009)
Bimodal bacteria	Bacteria observed to be present in either high or low abundances within a given population of individuals. The states may be driven by external factors and are not necessarily resilient to changes.	Lahti *et al.* (2014)
Bistable bacteria	Bacteria that have two alternative stable states of low and high abundance, and unlike bimodal bacteria, the states are able to resist change.	Faith *et al.* (2013); Lahti *et al.* (2014)
Common core microbiota	Community of microbes and their functions that are shared in majority of humans.	Qin *et al.* (2010)
Deterministic effect	Assembly of communities in a deterministic manner which is a result of environmental factors and interactions between community members.	Fukami and Nakajima (2011)
Human intestinal microbiome	The entire habitat (all microbes, their genomes and surrounding environment) in the human.	Marchesi and Ravel (2015)
Microbiota	The assemblage (collection and combination) of microorganisms present in a defined environment	Marchesi and Ravel (2015)
Minimal intestinal metagenome	The catalog of functions (coding capacity as assessed by metagenomics) involved in the homeostasis of the whole ecosystem, encoded across many species.	Qin *et al.* (2010)
Resilience	The capacity of a community to return to the original state after perturbation.	Paine, Tegner and Johnson (1998); Walker *et al.* (2004)
Stability	Tendency of the community to maintain a state of homeostasis and resist disturbances or to show resilience after disturbance.	Scheffer *et al.* (2001)
Stochastic effect	Assembly of communities as a result of unpredictable disturbance, dispersal or birth–death events.	Chase and Myers (2011)

coexisting community of microbes and their functions, which are characterized by the minimal intestinal metagenome, have co-evolved with the host for mutual benefits and are prevalent in the population, constitutes the common core (Jalanka-Tuovinen *et al.* 2011; Li *et al.* 2014). The core microbiota may include specific keystone species that are important for maintaining an efficiently functioning ecosystem, and whose gain or loss may have profound influence on ecosystem structure and function through their effect on other community members (Paine 1966). In the intestinal tract ecosystem, examples of keystone species include bacteria that are part of the common core. Their functional capabilities include for instance breakdown of complex carbon sources to support the growth of the other core members (Ze *et al.* 2013; Trosvik and Muinck 2015). In the following section, we will discuss both the phylogenetic core and the minimal intestinal metagenome.

Phylogenetic core

Several studies focused on defining a human intestinal core microbiota in terms of phylogenetic composition (Hamady and Knight 2009). Longitudinal surveys of fecal bacterial 16S ribosomal RNA (rRNA) sequences in individuals for extended periods of time of up to more than 10 years have revealed that a significant fraction of bacterial phylotypes is continuously present in the large intestine indicating that it comprises a stable individual core in healthy adults (Faith *et al.* 2013; Rajilić-Stojanović *et al.* 2013). Cross-sectional comparisons of the fecal microbiota in a large population cohort have indicated the presence of a prevalent core microbiota, which comprises those bacterial taxa that can be detected in the majority of individuals (Zoetendal, Rajilić-Stojanović and De Vos 2008). In contrast, the individual core microbiota includes those taxa that are frequently detected within a particular individual; this can overlap with the common core, but also include individual-specific features.

Several studies have aimed to characterize the composition of the common core but their comparison is difficult since different criteria were used (Table 2). The discrepancy in categorizing the common core microbiota could be partially attributed to methodological differences in determining microbiota composition and defining the core. Importantly, the depth of sequencing and analysis, number of samples and taxonomic resolution are the factors that affect the analysis.

The aforementioned issues pertaining to the analytical definition of core microbiota have been addressed by using the HITChip (Human Intestinal Tract Chip), a phylogenetic microarray targeting 16S rRNA gene sequences of bacterial taxa that are reported to occur in the human intestinal tract (Salonen *et al.* 2012). This phylogenetic microarray represents a measurement platform with high reproducibility, robustness and dynamic range, allowing the incorporation of both abundant and rare taxa in microbiota profiling. However, while capturing the vast majority of intestinal microbial species, these and other phylogenetic microarrays lack the ability to detect novel taxa

Table 2. Overview of studies that aimed at identifying the compositional and functional core.

Sr. No.	Core Compositional	Functional¶	Number of subjects	Criterion—other comments	Study/ year
1	No	Yes	154	Presence of OTU in all individuals—both obese and lean subjects included	Turnbaugh *et al.* (2009)
2	66 OTUs	No	35	Presence of OTU in more than 50% individuals	Tap *et al.* (2009)
3	15 genus-like groups	No	10	The study used phylogenetic microarray for the first time to demonstrate the common core	Rajilić-Stojanović *et al.* (2009)
4	19	No	4	Low number of subjects	Claesson *et al.* (2009)
5	406 in elderly subjects (at least 65 yr). 18 in subjects between 18 and 58 yr	No	127	Criterion was presence of OTU in more than 50% individuals. Included ageing subjects	Claesson *et al.* (2011)
6	6% (V1–V3) to 8% (V3–V5)	No	327	Criterion was presence of OTU in at least 95% of individuals	Huse *et al.* (2012)
7	Not studied	Yes	3	Criterion was proteome features present in all subjects	Kolmeder *et al.* (2012)
8	Variable	No	104	Depending on the cut-offs selected for ubiquity and abundance	Li, Bihan and Methé (2013)
9	75 species	294 110 genes	124	The most common species were those present in ≥90% of individuals with genome coverage >1% Common genes are those present in >50%	Qin *et al.* (2010)
10	290 phylotypes	No	115	Core size depends on depth of analysis. Present in all individuals (phylogenetic microarray technology)	Salonen *et al.* (2012)
10	288 phylotypes	No	9	Present in all individuals at a detection threshold of 0.03% (phylogenetic microarray technology)	Jalanka-Tuovinen *et al.* (2011)
11	22 OTUs	No	64	Observed at an average frequency of occurrence higher than 90%	Zhang *et al.* (2014)
12	43 OTUs	No	20	Present in 15 out of 20 individuals	Nam *et al.* (2011)
13	9 genera, 30 OTUs	No	314	9 genera present in all and at OTU level shared by at least 90%	Zhang *et al.* (2015)
14	17 genera	No	4000 (Dutch, Belgian, UK and USA)	Prevalence threshold was 95%. At varying low mean abundance the number of core genera detected was higher	Falony *et al.* (2016)
	14 genera		4000 plus 308 samples from Papua New Guinea Peru, and Tanzania		
	35 genera		In 1106 individuals from the Belgian Flemish Gut Flora Project cohort		

that can be assessed by next-generation sequencing techniques (Hazen *et al.* 2010; Roh *et al.* 2010; Tottey *et al.* 2013). Previously, the HITChip-based analysis of >1000 rare and abundant bacterial phylotypes from 130 genus-level groups in 115 adults revealed a common core of 290 phylotypes (Jalanka-Tuovinen *et al.* 2011; Salonen *et al.* 2012). We extended this methodology to analyze the common core in a cohort of 1006 non-compromised Western adults (Lahti *et al.* 2014). This provided a global view of the common core that revealed bacteria related to *Faecalibacterium prausnitzii*, *Oscillospira guillermondii* and *Ruminococcus obeum* as the top three taxa shared by all adults (Fig. S1, Supporting Information). Previously, we reported that the method used for DNA extraction affected the microbiome analysis and that mechanical extraction is to be preferred, as further confirmed by

the International Human Microbiome Standards analysis (Salonen *et al.* 2010; Santiago *et al.* 2014). Hence, we separately analyzed the samples processed using a mechanical or enzymatic DNA extraction protocol. While no major difference in the inclusion or exclusion of major core bacteria was observed, differences in their ranked cumulative fractional abundances were observed (Fig. S2, Supporting Information). Limiting our analysis to the subset of 401 samples obtained by mechanical extraction using repeated bead-beating revealed that bacteria related to *F. prausnitzii, R. obeum* and *Subdoligranulum variabile* to be the top three taxa shared by all adults. Moreover, we found that 34 out of the 130 genus-like groups targeted by the HITChip are shared in 95% of the subjects at the minimum detection threshold of 0.1% relative abundance, with the highest prevalence being observed for bacteria belonging to the order *Clostridiales* (Fig. 1; see Supporting Information for methods). This estimate is similar in number to the recently published data by the Belgian Flemish Gut Flora Project (FGFP) on 1106 individuals at 95% prevalence (Falony *et al.* 2016). However, in the same study, additional inclusion of the subjects from the LifeLines-DEEP study cohort (Dutch nationals), as well as from UK and US studies, resulted in a reduced core consisting of 17 genera that could be observed in both cohorts and there was no specific threshold on relative abundance for identifying core bacteria. In comparison, our study cohort included subjects from across mainland Europe, UK/Ireland and the USA (see Supporting Information). The lower estimates of the core size in the FGFP cohort could be partially explained by technical issues, including differences in DNA extraction methods, variations in sequencing depth or 16S rRNA gene primer sequences. However, at genus level, the reported core taxa from these two studies are largely consistent (Table S1, Supporting Information). Furthermore, reducing the stringency in the prevalence threshold in our data set to 80% and 50% with a constant detection threshold of 0.1% resulted in an increased size of the common core from 34 to 44 and 55 genera at 80% and 50% prevalence, respectively (Fig. 1 and Table S1, Supporting Information). Thus, half the population studied here shared 42% of the 130 genus-like groups investigated. This further supports previous observations that the definition of the core microbiota is sensitive to the abundance and prevalence thresholds (Salonen *et al.* 2012). In addition, there is a large amount of shotgun metagenomics data available in public databases which in some cases can identify species and led to the metagenome species concept defining not yet cultured and genomically sequenced taxa (Sunagawa *et al.* 2013). This metagenomic approach is not affected by primer or PCR bias but to some extent is limited by the depth of sequencing and choice of DNA extraction method. Therefore, we further investigated the largest available metagenome catalog of 1267 human fecal samples to identify the core bacterial genera (Li *et al.* 2014). At a detection threshold set at 0.1%, only *Bacteroides* spp. were detected to be present in >90% of the samples (Table S1, Supporting Information). In order to overcome the influence of lower sequencing depth, we reduced the abundance threshold by 10-fold to 0.01% (still a conservative threshold) and identified eight genera namely *Bacteroides, Eubacterium, Faecalibacterium, Alistipes, Ruminococcus, Clostridium, Roseburia* and *Blautia* in more than half of the studied population (Table S1, Supporting Information). While a comparison of technologies is not the aim of the current review, we would like to highlight that the core microbiota includes a limited number of taxa that are commonly observed, irrespective of the technology used. As sequencing costs decline and new bioinformatics tools are being developed, shotgun metagenomics can be expected to become a standard in microbiome research and will lead to more uniformity between studies for comparisons. Hence, it will be imperative to revisit the core microbiota analysis in the future for more conclusive evidence. Studies incorporating strain-level comparison and identification of genomes without a reference genome will provide an even more detailed picture of the members, dynamics and functional aspects of the core microbiota (Qin *et al.* 2010; Li *et al.* 2014; Scholz *et al.* 2016). We hypothesize that different phylogenetic groups, which share important core functions in the intestinal ecosystem, co-exist and are part of the core. Analysis of the cumulative fractional abundance indicated that the common core bacteria are among the most dominant taxa in the Western adult population, including bacteria related to *F. prausnitzii, R. obeum, S. variabile*, *Prevotella melaninogenica* and *Oscillospira guillermondii* (Fig. S2, Supporting Information). Cultured representatives for the vast majority of the common core bacteria are available and have sequenced genomes with information regarding their general metabolic traits and their association with human health. To this end, an extensive survey of literature was done to identify the general metabolic traits of the common core genus-like groups, and information for a subset discussed in more detail in this review is highlighted in Table 3. For information on other common core genus-like groups, see Table S2 (Supporting Information). We observed that there is a lack of in-depth understanding of the physiology of most of the core bacteria and their potential role in improving the stability of the intestinal microbiome.

Minimal intestinal metagenome

The human intestinal microbiome is a functional organ, with complex overall effects on host health. Therefore, it is crucial to identify functions commonly associated with the microbiota in health and disease. Whole shotgun metagenomics is widely used for identifying functional potential of the intestinal microbiome. Initially, the metagenomic core was analyzed based on the DNA isolated from fecal samples of 18 individuals with European ancestry (Turnbaugh *et al.* 2009). Despite the limited sample size, this analysis suggested the presence of a minimal intestinal metagenome that was shared by almost all subjects in this study. In a first comprehensive metagenome study, the functional similarities of the fecal metagenome of 124 European individuals with 3.3 million unique open reading frames were reported and indicated that on average 38% of an individual's total gene pool is shared with others (Qin *et al.* 2010). In a recent study, the coding capacity of the large intestinal microbiome was addressed in further detail by studying the metagenome of 1070 individuals from three continents, i.e. Europe, America and Asia (Li *et al.* 2014). This study, which included both healthy and compromised subjects, identified around 9.9 million unique microbial genes within the intestinal microbiome, and the analysis indicated that this estimate is close to the saturated coverage of core gene content and functions. In case of the intestinal ecosystem, the minimal functional core can be expected to also contain more ecosystem-specific pathways, such as those involved in resistance to bile and ability to grow in an anoxic environment using fermentation or anaerobic respiration with terminal electron acceptors other than oxygen. In the first large-scale metagenomic study, more than 1000 clusters of genes that encode vital functions necessary for survival in the large intestine were identified (Qin *et al.* 2010). This would suggest that the genomes of core bacterial taxa would have evolved toward high

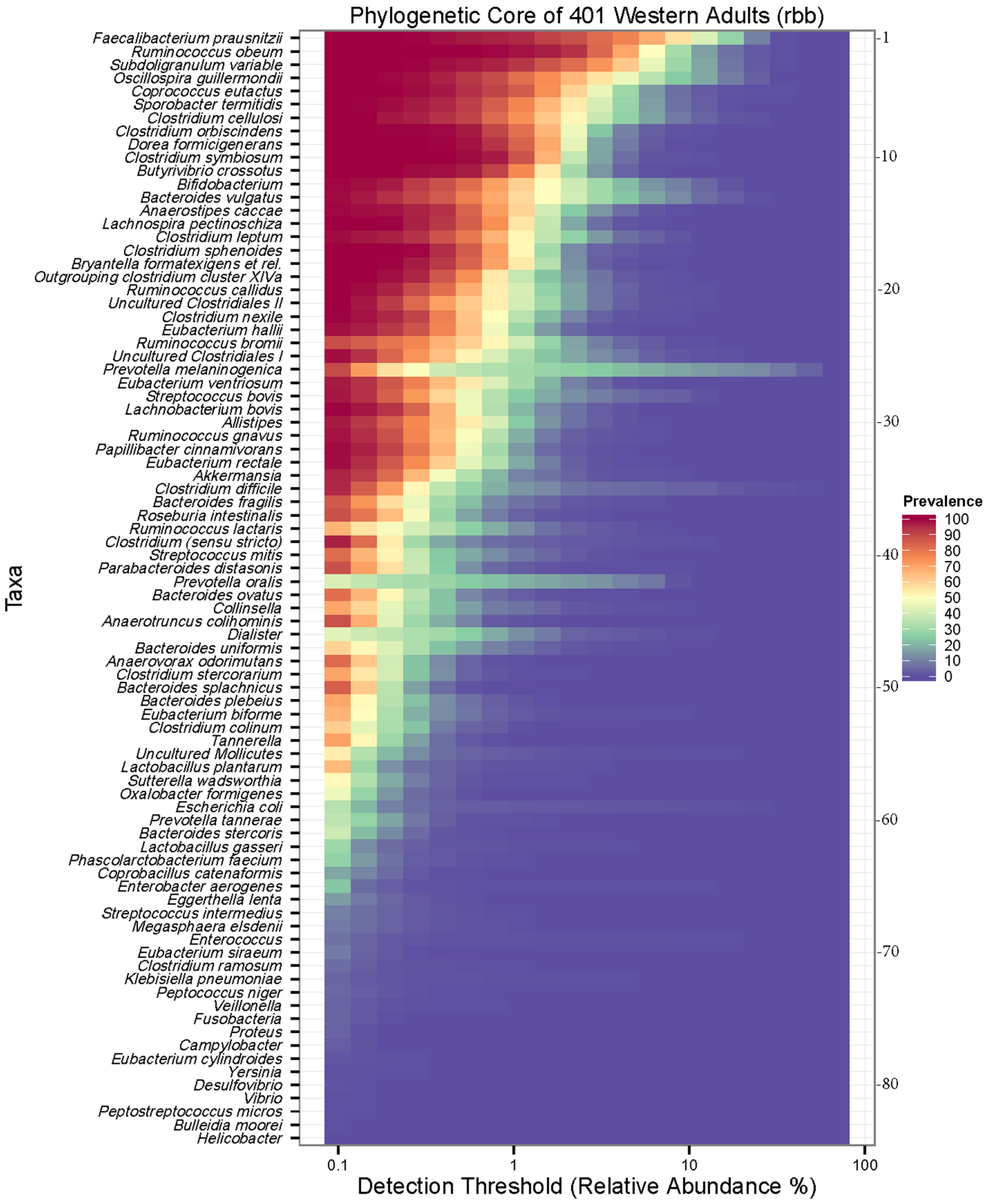

Figure 1. Bacterial phylogenetic core in the human large intestinal microbiome. We use data and analysis methodology from our previous studies (Jalanka-Tuovinen *et al.* 2011; Salonen *et al.* 2012). The data set was filtered based on DNA extraction method i.e. we included only the samples processed with the repeated bead beating method (rbb) which has been shown to outperform other methods (Salonen *et al.* 2010).

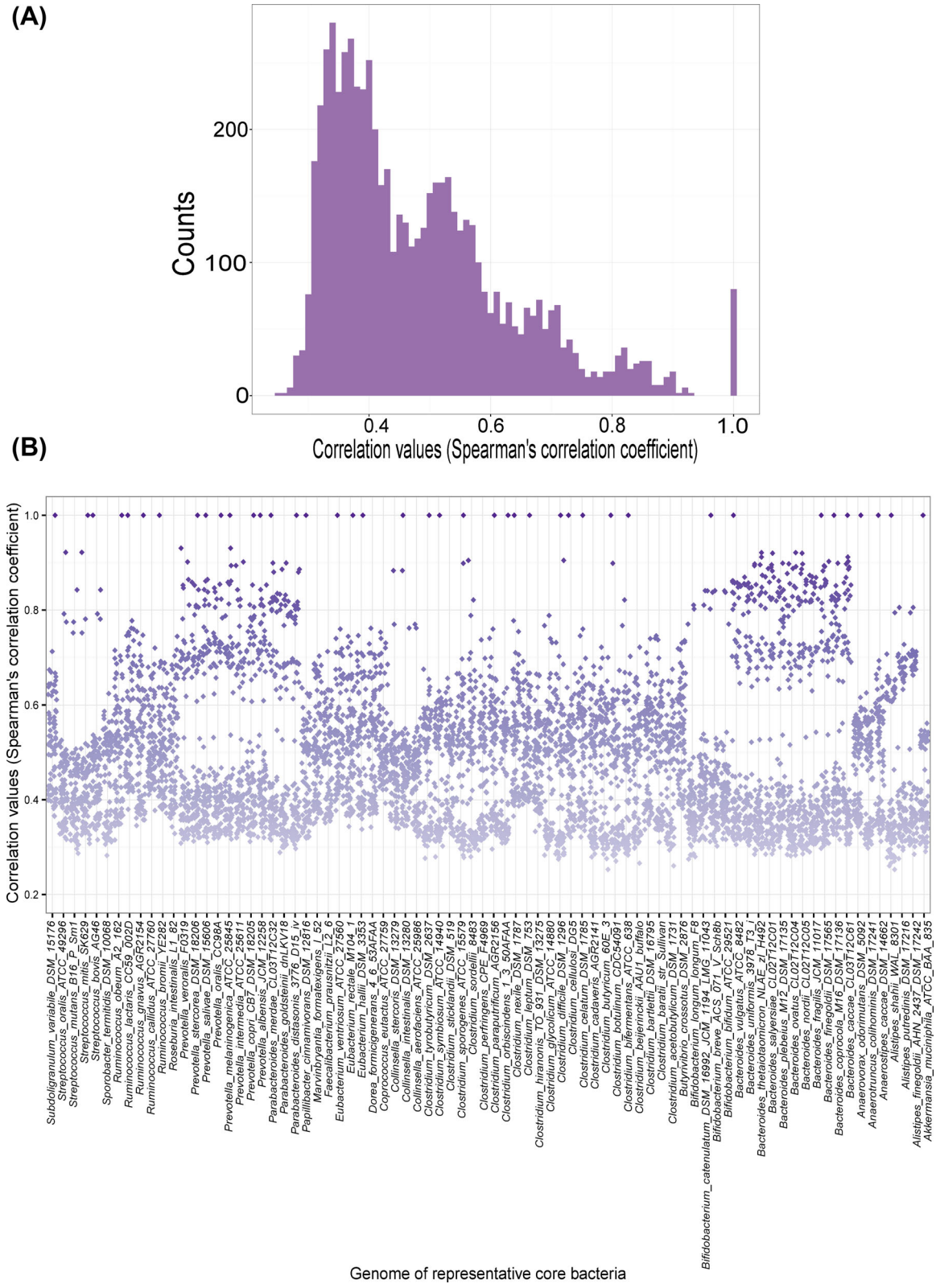

Figure 2. Spearman correlation coefficient for KO profiles for 80 genomes representative of the top 50 genus-level bacterial taxa including the phylogenetic core. (**A**) The distribution of Spearman correlation values in the core taxa. (**B**) The representative core bacterial genomes and their correlation to other genomes (each point depicts an independent genome correlated to genome on y-axis). The genomic data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community and were accessed using the IMG system.

Table 3. Selected core genus-like taxa in human large intestine. Prevalence, general metabolic trait and health associations of few key taxa in the human intestine. Microbiota profiling was done as described previously (Lahti *et al.* 2014). The analysis is based on 130 genus-like groups as defined by (Rajilić-Stojanović *et al.* 2009). For a more detailed information on all the common core genus-like taxa can be found in the supplementary Table S2.

Sr. No.	Genus-like taxa	Prevalence	General metabolic trait	Health association(s)
1	*Faecalibacterium prausnitzii*	100%	Produce butyrate, formate and lactate (Duncan *et al.* 2002)	Decreased abundance in Crohn's disease and colon cancer (Kang *et al.* 2010; Chen *et al.* 2012)
2	*Bifidobacterium*	99.50%	Produce lactate and acetate by utilizing various oligosaccharides (Turroni *et al.* 2008)	Widely used in probiotic preparations for health benefits (Turroni *et al.* 2008)
3	*Akkermansia*	96.26%	Dominant mucin degrader (Belzer and de Vos 2012)	Indicator of a healthy metabolic profile in humans (Dao *et al.* 2016)
4	*Prevotella melaninogenica*	89.53%	Acetate producer (Wu *et al.* 1992)	One of the tipping elements (Lahti *et al.* 2014)
5	*Ruminococcus bromii*	88.53%	Amylolytic activity (Ze *et al.* 2012)	Keystone species and healthy effects via breakdown of resistant starch (Ze *et al.* 2012)
5	*Bacteroides fragilis*	86.28%	Few strains have ability to degrade mucin (Tailford *et al.* 2015)	Anti-inflammatory effects and also opportunistic pathogen (Sansonetti 2011)

functional redundancy. To assess whether the common core observed in this study also demonstrated functional redundancy, we analyzed the representative genomes of the top 50 bacterial taxa that are part of the phylogenetic core (Fig. 1). Since our analysis was performed at genus level, we included known intestinal genomes from different species targeted by the HITChip which resulted in selection of 80 bacterial genomes representative of 50 genus-like taxa (Table S3, Supporting Information) (Rajilić-Stojanović *et al.* 2009). We first identified the KEGG orthologs (KO) abundances for each of the genomes using the Integrated Microbial Genomes (IMG) system and calculated the Spearman correlation coefficient (Markowitz *et al.* 2012) (Figure 2, see Supplementary data for details). From the inferred network, we observed that bacteria belonging to the phylum *Bacteroidetes* have high functional redundancy, whereas the phylum *Firmicutes* was comprised of a large number of more functionally diverse core bacteria (Fig. 3). Recently, analysis of the gyraseB encoding gene (*gyrB*) in fecal samples from humans and related primates (wild chimpanzees, bonobos and gorillas) indicated that *Bacteroides* and *Bifidobacterium* spp. have cospeciated with their mammalian hosts over thousands of generations suggesting strong symbiotic association of these taxa (Moeller *et al.* 2016). This would indicate that the host may also play a role in selecting functionally redundant species, which results in high competition for resources in the ecosystem but at the same time leads to higher ecosystem stability, which ultimately benefits the host (Coyte, Schluter and Foster 2015). However, the common core also includes taxa with specialized functional roles. A striking example is *Ruminococcus bromii* that was previously reported as a keystone species for its ability to degrade resistant starch and supporting growth of other bacteria capable of utilizing glucose, maltose, panose and isomaltose for growth (Ze *et al.* 2012). A similar role of providing simple carbon sources and amino acids for supporting growth of microbes in the vicinity of the mucus layer can be played by degraders of mucin, another important food source in the colon. The abundant representative of the *Verrucomicrobia*, *Akkermansia muciniphila*, another core member (Fig. 1), is considered a keystone species in this process and is capable of using mucus as sole carbon and energy source, while signaling to the intestinal mucosa (Belzer and de Vos 2012; Everard *et al.* 2013; Derrien, Belzer and de Vos 2016).

Metagenomic sequencing data can provide insights into the function, taxonomic position and strain-level diversity of both known and not yet cultured species (Sunagawa *et al.* 2013; Li *et al.* 2014; Nielsen *et al.* 2014; Jeraldo *et al.* 2016). However, the active functions within the intestinal microbiome cannot be identified based on DNA sequencing data alone but require analysis of transcripts and proteins. To this end, several studies have addressed the active functional capacity of the intestinal microbiota at the RNA and protein level, supporting the presence of an active functional core (Verberkmoes *et al.* 2009; Erickson *et al.* 2012; Kolmeder *et al.* 2012, 2015). Metatranscriptomics analysis of fecal samples from 10 American adults revealed alterations in gene-expression profiles after changes in dietary intake (David *et al.* 2014). Similarly, metaproteomic comparison of 29 non-obese and obese adults has shown larger differences in the active functional bacteria between the two groups than those observed at the compositional level (Kolmeder *et al.* 2015). While these studies are a step toward functional understanding of the human microbiome, the field is still young. Improved statistical techniques and clinically relevant predictions will play a key role in a wider adoption to identify changes of functional features characteristic of different disease states.

CLUSTERS, CONTINUOUS GRADIENTS AND ALTERNATIVE STABLE STATES

Several approaches have been employed to identify overarching community types, as well as alternative stable states in more specific taxonomic groups (Arumugam *et al.* 2011; Holmes, Harris and Quince 2012; Ding and Schloss 2014; Lahti *et al.* 2014). Moreover, longitudinal analysis of bimodal bacteria revealed temporal stability of the alternative states of low and high

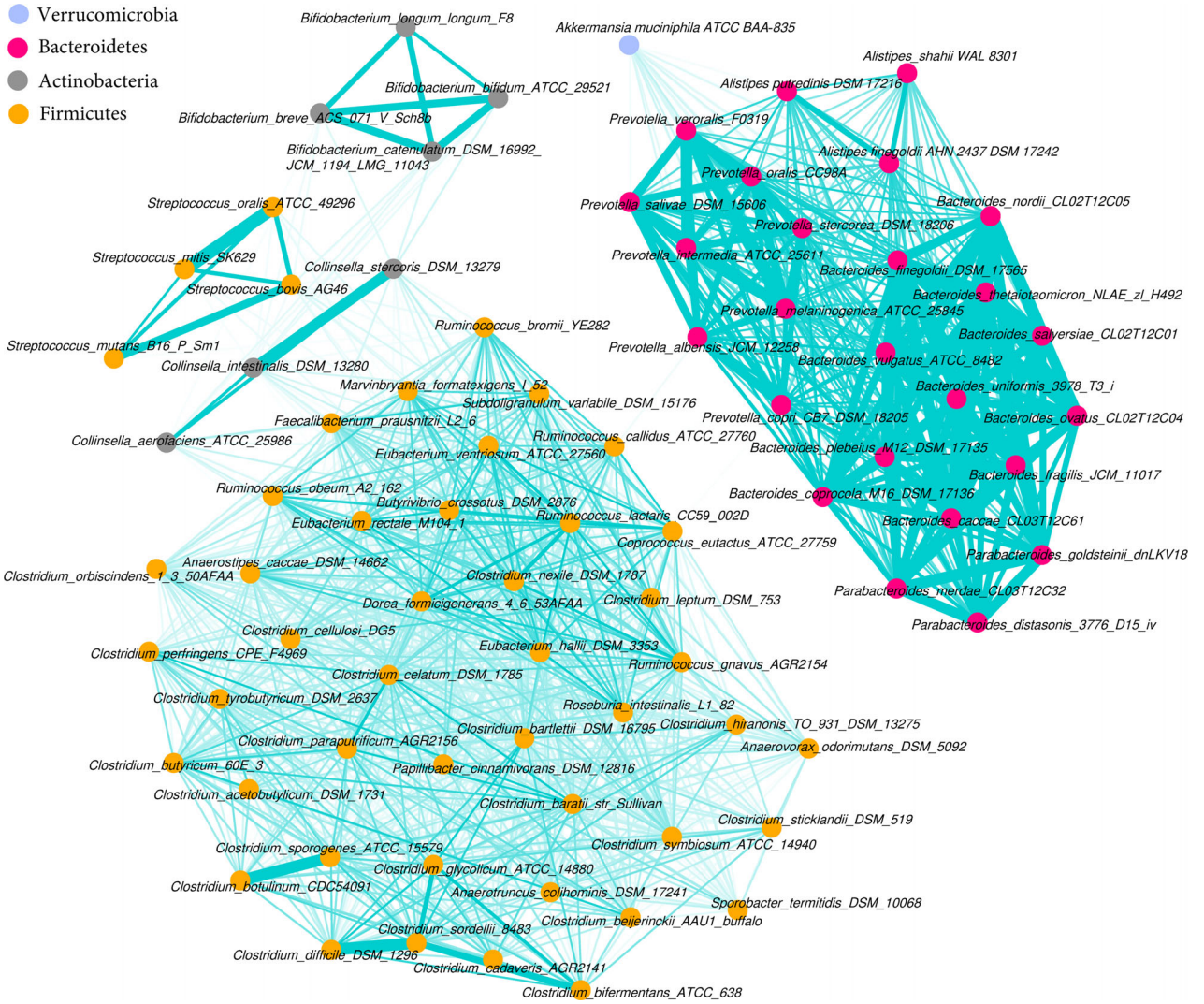

Figure 3. Functional correlation network of 80 bacterial genomes representative of the top 50 common core bacteria. The Spearman correlation matrix is represented as a network in which each genome is a node and each correlation an edge; the width of the edges is proportional to the magnitude of the correlation (the higher the correlation the thicker the edge line). The nodes with correlation coefficients below 0.5 are not connected, and genomes were placed by a graph 'spring' layout algorithm in qgraph (Epskamp *et al.* 2012). The genomic data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community and were accessed using the IMG system.

abundance, which are in some cases observable as ecosystem-level shifts (Lahti *et al.* 2014). One potential explanation for the observed bistability could be that these states are resilient to perturbations and stable over ecologically relevant timescales (Connell and Sousa 1983). Additionally, the transition from one stable state to an alternative stable state as a result of a disturbance by e.g. dietary change, may comprise a period which can be stable for a long time relative to the extreme end state, called the alternative transient state (Fukami and Nakajima 2011). In alternative transient states, the communities are in a variable, non-resilient state and vary in structure and/or function as a result of processes such as priority effects (time and order of species arrival), external conditions and deterministic processes (Fukami and Nakajima 2011). Thus, the intestinal bacterial community can be viewed as a landscape representing varying global and/or local levels of stability, species richness and diversity (Arumugam *et al.* 2011; Koren *et al.* 2013; Thaiss *et al.* 2014; Zoetendal and de Vos 2014). In this context, the core bacteria and their abundances can be considered as potential biomarkers that may indicate changes in the intestinal ecosystem.

Alternative community states

In an early approach to detect ecosystem-level structures in the intestinal microbiome, deep metagenome analysis suggested the presence of three clusters, termed enterotypes and characterized by co-occurring taxa named after their dominant bacterial groups i.e. *Bacteroides*, *Prevotella* and *Ruminococcus* (Arumugam *et al.* 2011). One can argue whether it is possible to infer community assembly rules for the complex and highly dynamic intestinal ecosystem based on discrete alternative community types or whether the intestinal microbiome should rather be viewed as a continuous dynamic ecosystem, which is in constant flux but existing in a microbiota–host homeostatic state (Jeffery *et al.* 2012; Knights *et al.* 2014). Recently, it was reported that the enterotype-like clustering observed in ordination analysis could be influenced by the

initial abundances of *Prevotella* and *Bacteroides* in the samples that were analyzed (Gorvitovskaia, Holmes and Huse 2016). Moreover, it has been demonstrated that high levels of *Prevotella* and *Bacteroides* can co-exist in the host, suggesting that these types could be complementary rather than mutually exclusive (Lahti *et al.* 2014). In addition, a separate study showed that removal of these two taxa from analysis did not result in differentiation of communities into clusters, suggesting that the dominance of *Prevotella* or *Bacteroides* did not represent underlying differences in community structure (Gorvitovskaia, Holmes and Huse 2016). However, that study also found that the dominance of *Prevotella* or *Bacteroides* correlated well with dietary habits and lifestyle. Thus, it was suggested that the abundance of *Prevotella* and *Bacteroides* might be considered as a 'biomarker' of diet and lifestyle instead of using the term enterotypes (Gorvitovskaia, Holmes and Huse 2016). A different approach to clustering and classification of samples based on microbial profiling data uses a probabilistic approach, called the Dirichlet multinomial mixture (DMM) models (Holmes, Harris and Quince 2012; Ding and Schloss 2014). This approach found evidence for the presence of alternative community configurations in the human intestinal microbiome regardless of the extensive intra- and interpersonal variations (Holmes, Harris and Quince 2012; Ding and Schloss 2014).

Ecologists have also applied graph theory-based approaches to identify species interactions networks and modularity. In most studies, the co-occurrence and co-abundance network have been inferred from correlation-based and other similarity measures (Weiss *et al.* 2016). One such attempt incorporated sparse regression with a number of similarity measures to predict microbial co-occurrence and co-exclusion relationships (Faust *et al.* 2012). It was observed that the microbial community is organized into microcommunities which would indicate a trade-off between certain combinations of microbes that may represent alternative communities (Faust *et al.* 2012). Recently, other tools have been developed to handle sparse compositional data (Friedman and Alm 2012; Gevers *et al.* 2014; Kurtz *et al.* 2015). Compared to the standard correlation analyses, these new methods take better into account the sparsity, compositionality and high dimensionality of the taxonomic networks. One example is the use of CCREPE to identify Crohn's disease associated community network where it was observed that proinflammatory bacteria dominate and co-exclude other potentially beneficial taxa (Gevers *et al.* 2014). This seems to be an example of a community shift. A similar observation of variable modularity in microbial interaction network was made in inflammatory bowel disease (IBD) patients and non-IBD controls (Baldassano and Bassett 2016). At the population level, the two states had a different modular structure and highlighted the different community memberships. However, it is vital to remember that the tools and models for identifying co-occurrences and modularity in microbiome data still need improvement (Weiss *et al.* 2016). In all cases, these studies are observational and do not include interventions and hence inferences on causality cannot be made (de Vos and de Vos 2012). In general, more comprehensive longitudinal analyses and intervention studies will be needed to establish causal relationships.

Continuous gradients and bistability

The observed dynamics of the large intestinal ecosystem suggests the possibility for transitions between alternative and potentially stable states, with diet being one of the major driving factors (Wu *et al.* 2011; Cotillard *et al.* 2013; Salonen *et al.* 2014; Falony *et al.* 2016). For instance, it has been observed that a diet rich in proteins and animal fat is associated with the *Bacteroides* dominance, whereas carbohydrates have been linked to high abundance of *Prevotella* (Wu *et al.* 2011). The extremes of dietary habits point toward a *Prevotella*/*Bacteroides* trade-off driven by diet and suggesting a gradient in relative abundances between the two extreme states (high and low abundance) (Kelsen and Wu 2012). The joint population frequencies (represented as peaks and valleys) of the bistable taxa, *Prevotella* and *Bacteroides* exhibit a landscape distribution in the Western adult population (Fig. 4A). This highlights the population-level variation in these key taxonomic groups. These two taxa exhibit high degree of functional redundancy as based on their genomic content when compared to functions shared between populations belonging to the *Firmicutes* or *Bifidobacterium* spp. (Fig. 3). Previously, ecosystem-level models based on metagenomic and genomics data indicated that the habitat filtering (selection pressure inflicted by the habitat) may have greater impact than functional independence or cooperation on the community composition and thus, may result in selection of functionally redundant species (Levy and Borenstein 2014). It has also been proposed that the stability of intestinal microbiota is affected more by competing species than cooperating species (Coyte, Schluter and Foster 2015). A new hypothesis emerged from the analysis of genome-based models that showed the module-derived functional redundancy to be reduced in the *Bacteroides* spp. as compared to other genera, potentially linking this to a decreased resilience to perturbations (Vieira-Silva *et al.* 2016). Future studies aimed at mechanistic understanding of the interaction between these and other highly abundant or bistable taxa in the community and their potential role in ecosystemic transitions could provide the clues for microbiota modulation for instance based on dietary, antibiotic or other interventions.

To assess the dynamics of the large intestinal microbiota, it is necessary to study microbiota composition over different time scales and investigate the influence of perturbations. Compared to cross-sectional studies, analysis of time-series can provide information on intraindividual variations. Longitudinal analysis of larger populations gives insights into dynamics and stability of microbiota and could be informative in identifying key species that may play a role in ecosystem shifts (Faust *et al.* 2015). By applying a discrete time Lotka-Volterra model to characterize bacterial interaction networks, it was observed that a high abundance does not necessarily imply a large role in intestinal microbiota stability (Fisher and Mehta 2014). The identified keystone species (*Bacteroides fragilis* and *B. stercosis*) in this study had moderate abundances but showed disproportionate influence on the interaction network. However, this study was limited by the number of individuals (two) included in the study. Variations of the Lotka-Volterra model have been used widely in ecology for the understanding of two species interactions. It is well known that the use of antibiotics may have a large impact on the microbial community and makes it prone to invasion by pathobionts (Bokulich *et al.* 2016). A combination of Lotka-Volterra modeling and regression analysis in antibiotic-treated mice predicted that a microcommunity network was important in protection against *Clostridium difficile* invasion (Stein *et al.* 2013). It was observed that a compromised community showed a catastrophic shift into an alternative state, which was stable and did not revert back to the initial community state after removal of the perturbations. Future studies applying these and other models on larger human cohorts may give better insights and aid in understanding ecology of the large intestinal microbiome.

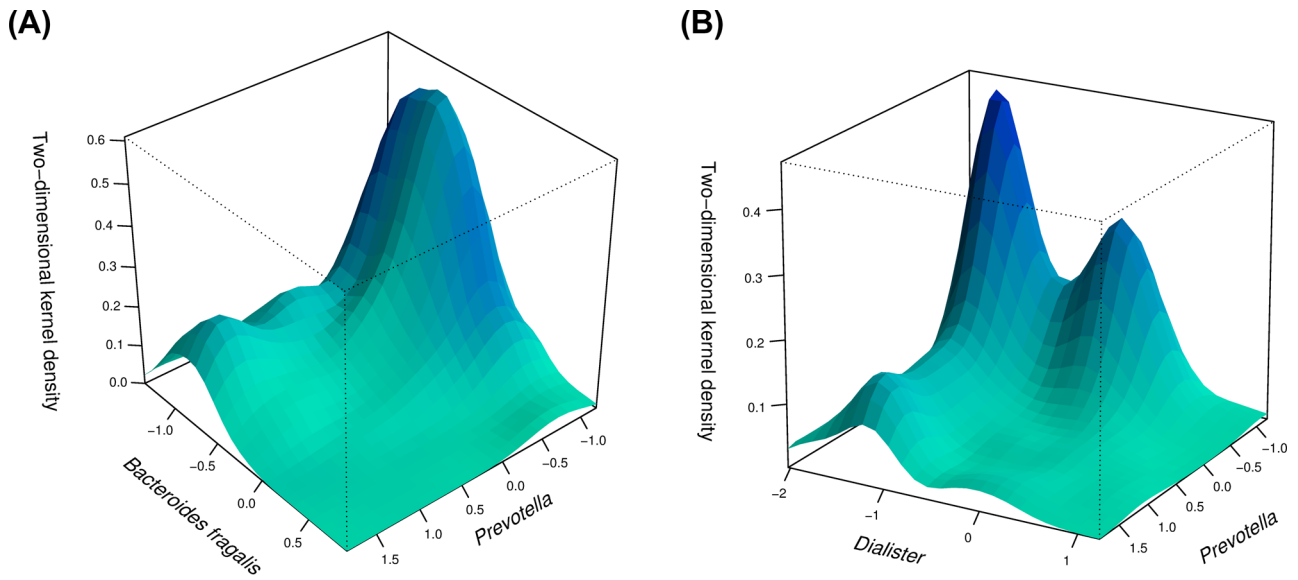

Figure 4. Two-dimensional kernel density (2D-kde) estimates. The abundance of bacteria (log10 transformed relative abundance data) mapped onto the x- axis and y-axis respectively, and the z-axis represents the kernel density estimate, in a 3D perspective plot showing the joint population frequencies of these two taxa demonstrating features similar to a landscape. (**A**) Mapping of *P. melaninogenica* and *B. fragilis* illustrates two distinct peaks that represent low *B. fragilis* and high *P. melaninogenica* population and low *B. fragilis* low *P. melaninogenica* population. (**B**) Mapping of *P. melaninogenica* and *Dialister* illustrates three distinct peaks that represent low *Dialister* and high *P. melaninogenica* population; low *Dialister* and low *P. melaninogenica* population; high *Dialister* and low *P. melaninogenica* population. The phylogenetic microarray data were obtained from previous study (Lahti *et al.* 2014).

In the intestinal tract microbiota, there exists a dynamic state and consists of species with varying fluctuations in abundances (Caporaso *et al.* 2011; Flores *et al.* 2014; Thaiss *et al.* 2014). As there are thousands of species in the ecosystem, stability may arise in multiple states within a closed domain of possible alternative states also called 'basins of attraction' defined by a dynamic attractor (Scheffer *et al.* 2001; Rosenfeld 2008; Pepper and Rosenfeld 2012). The microbial community in a given individual may move from one attractor to another over time and may include transiently occurring species from external sources (e.g. food) (Fukami and Nakajima 2011; Lang, Eisen and Zivkovic 2014). In many studies, single time point fecal samples are analyzed and these may represent alternative transient states and consequently explain the high individuality observed in many studies (Flores *et al.* 2014). However, personalized patterns in the intestinal microbiota were detectable over 10 years of time, as shown by long-term monitoring of five healthy subjects (Rajilić-Stojanović *et al.* 2013). Dense sampling of the human intestinal microbiota has revealed diurnal oscillations in relative abundance of almost 10% of all bacterial taxa and revealed a repetitive pattern of fluctuations in microbiota community configurations specific for a given time of the day (Thaiss *et al.* 2014). These fluctuations highlight the dynamic nature of the intestinal microbiota and indicate that there exists low and high abundance states for most bacteria. When put in perspective of the alternative transient states, these observations reveal an interesting feature of the intestinal microbiota. It is possible that the daily factors such as diet result in changes in the microbiota structure and composition (introduction of transient bacteria through food and water) and thus result in alternative transient states. This has been experimentally verified by small studies of extreme diets (plant-based diet or animal-based diet) and a diet swap study of African Americans and Native Africans (David *et al.* 2014; O'Keefe *et al.* 2015). There can be several possible paths of alternative transient states that may reach in one of multiple states within a limited set of attractors. However, if there is a major shift in dietary habit, this might push the community on an alternative transient path that ultimately results in a catastrophic shift in the community. Investigation of individual bacterial groups revealed bistability (i.e. alternative states of either high or low relative abundance) and identified six genus-level groups that were not only bimodal in their population frequencies but also exhibited bistability, i.e. being predominantly present at either high or low relative abundance with less frequent observations and a reduced temporal stability of the intermediate abundances (Lahti *et al.* 2014). The intermediate abundance range (tipping point) exhibited reduced temporal stability within subjects (Lahti *et al.* 2014). These bistable groups varied relatively independently and hence were termed 'tipping elements'. At a community level, the corresponding compositional states could be described as specific combinations of such bistable tipping elements. Similar to *Prevotella melaninogenica and B. fragilis* distribution, we were interested in observing the population frequencies of highly abundant bistable bacteria *P. melaninogenica* and a lower abundance bistable taxon such as *Dialister* spp. in the cohort of Western adults. We used these examples to illustrate the alternative stable states mapping of the two-dimensional kernel density estimates for bacteria related to *P. melaninogenica* and *Dialister*. This analysis revealed three areas with high kernel density (Fig. 4B). The less abundant *Dialister* group can exist in its high and low abundance states with *P. melaninogenica* low and high abundance states. Previously, the longitudinal assessment of bistability revealed that *Prevotella* groups exist in highly bistable states and that their abundance levels are more stable in individuals over 3 months compared to other bistable taxa (Lahti *et al.* 2014). Furthermore, it was observed that the intestinal microbiota composition of individuals with bistable taxa closer to the tipping point is more variable in time (Lahti *et al.* 2014). This indicates that the two extreme states are resilient,

and changes in the ecosystem are more pronounced when bistable taxa have relative abundances closer to their tipping point.

POTENTIAL IMPLICATIONS OF COMMUNITY CHARACTERISTICS FOR MICROBIOME MODULATION STRATEGIES

In the past few decades, research investigating the ecology of the intestinal microbiome has revealed various features, such as community assembly, temporal dynamics and stability and host covariates associated with intestinal microbiota (Table 4). The knowledge of positive, negative or neutral effects of bacteria on the ecosystem may help in identifying targets for therapeutic modulation. Additionally, features such as resilience of the ecosystem and effect of perturbations need to be understood in detail. In the modification of an ecosystem, perturbation (random, episodic or programmed events) plays a major role (Paine, Tegner and Johnson 1998). The type of perturbation will affect the assembly of the community and also play a role in determining the recovery state. For example, the low and high *Prevotella* states are highly stable and do not easily change over a short period of treatment (Lahti *et al.* 2014). Furthermore, the initial state of a bacterial community has been reported to affect its recovery after antibiotic treatment (Raymond *et al.* 2015). It has also been observed that a low calorie dietary intervention could be helpful for obese individuals with lower bacterial richness (Tap *et al.* 2015). Richness of the large intestinal microbiome is commonly associated with higher resilience (Lozupone *et al.* 2012). Resilience and stability are the key features of the intestinal microbiome that need to be addressed while attempting

Table 4. Representative studies investigating the ecology of the intestinal microbiome employing high-throughput sequencing approach (most recent in each category have been listed).

Sr. No.	Names of the study	Conceptual insights
1	Temporal variability is a personalized feature of the human microbiome (Flores *et al.* 2014).	Elucidates the temporal dynamics and stability of the microbiome including the human.
2	Moving pictures of the human microbiome (Caporaso *et al.* 2011).	First large-scale longitudinal sampling temporal dynamics and stability of the human microbiome.
3	Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis (Thaiss *et al.* 2014).	Address the diurnal changes in microbiota and identifies hourly fluctuations in some bacterial genera.
5	Cospeciation of gut microbiota with hominids (Moeller *et al.* 2016).	Provides support to the theory of cospeciation of human intestinal tract microbiota along with hominids.
5	Ecological modeling from time-series inference: insight into dynamics and stability of intestinal microbiota (Stein *et al.* 2013).	Temporal dynamics and stability and influence of perturbation on community dynamics and stability.
6	The long-term stability of the human gut microbiota (Faith *et al.* 2013).	Addresses the question of long-term stability of microbial community using novel high-throughput sequencing method.
7	Antibiotics, birth mode and diet shape microbiome maturation during early life (Bokulich *et al.* 2016).	Elucidated the community assembly in early life and effect of perturbation on shaping the microbiome.
8	Population-level analysis of gut microbiome variation (Falony *et al.* 2016).	Population level insights into intestinal microbial community structure and composition with analysis of large number of co-founding factors.
9	An integrated catalog of reference genes in the human gut microbiome (Li *et al.* 2014).	Population level insights into intestinal microbial community function.
10	Enterotypes of the human gut microbiome (Arumugam *et al.* 2011).	Differences in community types/clusters in human intestinal microbiome based on partitioning around the medoid method.
11	Dynamics and associations of microbial community types across the human body (Ding and Schloss 2014).	Differences in community types/clusters in human intestinal microbiome on similar lines of Enterotypes paper but using DMM models.
12	Linking long-term dietary patterns with gut microbial enterotypes (Wu *et al.* 2011).	Stability of *Prevotella* enterotype from the *Bacteroides* enterotype was tested.
13	Interpreting *Prevotella* and *Bacteroides* as biomarkers of diet and lifestyle (Gorvitovskaia, Holmes and Huse 2016)	Investigating data bias in detecting enterotypes and address the issues pertaining to it.
14	Microbial co-occurrence relationships in the human microbiome (Faust *et al.* 2012).	Community interactions networks, niche specialization and assembly of microbial community have been demonstrated.
15	The treatment-naive microbiome in new-onset Crohn's disease (Gevers *et al.* 2014).	Disease and healthy interaction network with respect to Crohn's disease are elucidated.
16	Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and IBD (Greenblum, Turnbaugh and Borenstein 2012).	Metabolic network modularity of microbiome in health and disease state.
17	Identifying keystone species in the human gut microbiome from metagenomic timeseries using sparse linear regression (Fisher and Mehta 2014).	A novel method for identifying the keystone species based on longitudinal studies is reported.
18	Ecology of bacteria in the human gastrointestinal tract—identification of keystone and foundation taxa (Trosvik and Muinck 2015)	Using network statistics and reverse ecology the authors identify foundation and keystone taxa in human intestinal microbiome.

modulation. Different alternative states may vary in both resilience (tendency of a system to return to a position of equilibrium when disturbed) and stability (tendency to maintain the state when subject to disturbance) (Walker *et al.* 2004). Detailed understanding and identification of potential markers of these features will aid in designing intestinal microbiome modulation strategies.

In various diseased conditions, there is a shift in the composition and function of the intestinal microbiota driven by specific bacteria (possibly pathobionts). These bacteria are responsible for stabilizing an alternative community state, which is unfavorable for host health by producing specific metabolites that deter growth/survival of other bacteria. For example, in an inflamed intestine, increased levels have been observed of proinflammatory bacteria, such as members of the *Enterobacteriaceae*, associated with increased levels of the proinflammatory lipopolysaccharide produced by this group of Gram-negative bacteria (Wang *et al.* 2007). The resulting inflammatory cascade includes an increased concentration of host-produced reactive oxygen species that hamper growth of indigenous anaerobic bacteria (Lupp *et al.* 2007). Thus, this aberration may represent an alternative stable state, the stability of which is maintained by an inflammatory milieu created by the host and influenced by members of the *Enterobacteriaceae*. Hence, the transition from higher densities of obligate anaerobes to proinflammatory facultative anaerobes would be critical for the establishment and/or maintenance of a state of increased inflammation. In such cases, a reduction in numbers of proinflammatory bacteria will be crucial for a shift toward an alternative state (may or may not be stable) no longer supportive of inflammation, and thus leading to re-establishment of anoxic conditions in the intestine. Along with the reduction in proinflammatory facultative anaerobes, it will be crucial to support the growth of bacteria capable of tolerating oxygen at low concentrations, such as lactobacilli and *Akkermansia muciniphila* that have anti-inflammatory effects for re-establishment of an anoxic milieu (Tien *et al.* 2006; Ouwerkerk *et al.* 2016).

The relative abundance of diagnostic indicator taxa, such as bacteria belonging to *Enterobacteriaceae* (increasing relative abundance) and *A. muciniphila* (decreasing relative abundance), was shown to indicate particular environmental conditions such as an inflamed intestinal milieu (Png *et al.* 2010). In addition, indicator taxa can be sensitive to perturbations and therefore might serve as an early warning sign of unhealthy shifts in the community. Such indicator taxa can be monitored as their abundance may reflect the efficacy of intervention strategies. Using the knowledge of drivers and indicators, it would be possible to detect diagnostic biomarkers, identify specific therapeutic microbes (single keystone species or mixed consortia) for treatment and suggest lifestyle changes (such as dietary habits) that will result in a 'healthier' intestinal bacterial community.

Evidence for the predictive nature of intestinal bacteria was recently presented when investigating obese subjects undergoing dietary interventions (Korpela *et al.* 2014). The responsiveness of the intestinal bacterial community to a specific dietary intervention was predicted by the baseline relative abundances of *Clostridium* clusters IV, IX and XIVa, and Bacilli. The members of several of the *Clostridium* clusters also include a number of the core phylotypes in the human intestine, most notably *Clostridium* cluster IV (*C. leptum* group, a major constituent of which is the *Ruminococcaceae* family) and *Clostridium* cluster XIVa (*C. coccoides* group, which resembles the *Lachnospiraceae* family) (Tap *et al.* 2009; Rajilić-Stojanović and de Vos 2014). Based on these findings, it is tempting to speculate that some core phylotypes may also serve as indicator species for responsiveness, and their relative abundance may have profound influence on the efficacy of a given intervention strategy. Another example is *A. muciniphila*, which is a part of the intestinal core, and has been reported to be indicative of a healthy metabolic profile. Furthermore, its abundance along with high gene richness was indicative of better clinical outcomes after calorie restriction diet in overweight/obese adults (Dao *et al.* 2016). Evidence supporting the importance of bacterial community structure in determining individuals' responses to dietary modulation (in ecological terms; ecological disturbance) was provided by the findings of Salonen *et al.* (2014). It was observed that the dietary intervention had little effect on the intestinal bacterial composition of non-responders who were characterized by higher initial bacterial diversity. Thus, a higher baseline bacterial diversity represents a more resilient community, and increased responsiveness would be characteristic of an unstable community. This highlights that the baseline bacterial diversity could be one of the potential markers in predicting responsiveness to dietary modulation. However, it is important to acknowledge the effect of other, more specific, factors such as relative abundance of certain bacteria, e.g. bistable bacteria, in determining an individuals' response to intervention. It will be interesting to investigate whether the bistable groups can serve as predictors for responsiveness or non-responsiveness to modulation strategies.

The functional core and the identification of bistability of specific metabolic processes could also provide clues for predicting efficacy of interventions. For instance, also the overall bacterial gene richness has been demonstrated to exhibit bimodal population frequencies (Le Chatelier *et al.* 2013). Western individuals have been observed to be separated into two groups based on gene count, i.e. low gene count (LGC) and high gene count (HGC), with low gene richness being associated with pronounced dys-metabolism and low-grade inflammation. Thus, LGC and HGC could represent two alternative states in which one state (LGC) represents a state that is indicative of increased risk for obesity (Cotillard *et al.* 2013). In this study, it was observed that the LGC group after dietary intervention had improved gene richness concomitant with improvement in the clinical phenotypes but showed little change in the inflammation status of these individuals. These functional alternative states, i.e. LGC and HGC, provide us with an opportunity to predict the efficacy of any approach used for modulation. This further highlights the importance of richness of the intestinal microbiome (both compositional and functional) as a factor in determining resilience to ecosystem changes. It is known that species richness determines the susceptibility to invasion by non-resident species and resilience to overall ecosystem disturbances (Levine and D'Antonio 1999). In case of the intestinal microbiome, resilience of a community with high functional richness adds an important dimension to our understanding of its complexity. Thus, there is a need for considering both species richness and functional gene richness during intervention studies aimed at microbiome modulation.

FUTURE PROSPECTS: APPLYING COMMUNITY LEVEL UNDERSTANDING FOR BENEFICIAL LANDSCAPING OF THE INTESTINAL MICROBIOME

In the past decade, numerous studies targeting the human intestinal microbiome have led toward remarkable advances in our understanding of the crucial role these microbes play in

human health. Recognizing the importance of this microbial community, the intestinal microbiome is often called a 'metabolic organ' signifying its crucial functional role which has an effect on our health status (Bocci 1992). Importantly, this metabolic organ represents a functioning ecosystem, which is governed by ecological mechanisms. At present, clinically meaningful evidence for the potential application of modulating the intestinal microbiota for therapeutic gain has created considerable interest and enthusiasm (Smits *et al.* 2013; Li *et al.* 2016). Treatment of metabolic syndrome and *Clostridium difficile* infection by ecosystem-level cleansing restoration via fecal microbiota transplantation (FMT) has demonstrated the potential for translating microbiota research to clinical practice (Li *et al.* 2016). While FMT is promising with proven efficacy in several cases, the underlying mechanisms that result in beneficial effects on host health are unclear and will need further refinement. One of the approaches is targeting specific group(s) of bacteria to bring about the desired changes in the intestinal microbiome. The state of a dynamic system may be manipulated by triggering a switch to an alternative state, for instance with a strong but temporally limited, transient perturbation ('pulse', antibiotics), or longer impact ('press', diet) (Costello *et al.* 2012; Faust *et al.* 2015). These transient perturbations can also be combined with introduction of single bacteria or groups of bacteria that support or inhibit growth of specific bacteria resulting in desired changes in composition and function of the intestinal microbiome.

Managing invasion or overgrowth of pathobionts

In order to manage invasions or overgrowth of pathobionts, we first need to identify phylotypes that are common residents and their relative proportions in the intestinal ecosystem. The study of common core phylotypes and core functions is important in this aspect as this would form the baseline knowledge of 'normal' inhabitants. Comparison of this core healthy state with diseased states will assist in identifying invasive species (potentially harmful species that are not part of the core) and/or pathobionts (resident microbes with pathogenic potential). For instance, during IBD *Enterobacteriacaeae* are known potential pathobiont species that affect the numbers of anaerobic intestinal bacteria as mentioned above. In such cases, the use of therapeutic microbes combined with specific dietary interventions can be used to support growth of core phylotypes capable of fermenting complex polysaccharides to produce short-chain fatty acids (SCFA) and gas (CO_2 and H_2), which results in decreased intraluminal pH to establish an anoxic environment (Tien *et al.* 2006; Damaskos and Kolios 2008). Other examples of pathobionts in the human gastrointestinal intestinal tract are *Helicobacter pylori, Fusobacterium* spp., *Desulfovibrio* and *C. difficile* (Biagi *et al.* 2010; Rowan *et al.* 2010; Castellarin *et al.* 2012; Kamada *et al.* 2013; Martin and Solnick 2014). In most cases, the common observation in disease cases is the overgrowth of these pathobionts. In mouse models of antibiotic-mediated *C. difficile* infection, it was observed that a few bacteria confer protection and imbalance in their populations increases susceptibility to overgrowth by pathobionts (Stein *et al.* 2013). These few bacteria included *Akkermansia*, *Coprobacillus* and *Blautia* spp. that are all part of the core and their inhibition by antibiotics was proposed to support overgrowth of *C. difficile* assisted by *Enterococcus* spp. in mice (Stein *et al.* 2013). Thus, it could be possible that the knowledge of common core phylotypes combined with the low and high abundance states of pathobionts could help in designing specific approaches for managing invasion by pathogens or increased relative abundance of pathobionts. These approaches could include changes in the diet that support higher growth of core phylotypes and functions such as butyrate production which inhibits pathogen colonization (Scott *et al.* 2014).

Adapting intervention strategies for modulating the microbiome

The outcome of a given intervention strategy will depend on the individual's response to certain modulation attempts. The response of the individual to a specific intervention can be predicted and monitored by observing overall community descriptors such as species and gene richness and diversity, as well as abundance/presence/absence of key indicator taxa that may reflect the efficacy of intervention strategies. Indicator taxa could include bistable bacteria, and their shift toward the tipping point could provide clues for transition from diseased to healthy alternate stable states. Studying the transition between low and high abundance states or vice versa with an intermediate tipping point could provide clues for the critical time point for ecosystem restoration by modulating the microbiome toward a different, possibly healthier state. It is also possible that the shift from a diseased state may not necessarily lead to a healthy state but rather to a state different from either of the states. These alternative scenarios may be dependent on the strength of perturbation that resulted in shift to a diseased state. In any case, monitoring of microbiome composition and its dynamics over time should be incorporated in intervention strategies.

Maintenance of a healthy intestinal microbiome landscape throughout life span

Human health is interlinked with the human body and its associated microbiome, and thus, maintaining a healthy microbiome is essential. The characteristics of a healthy community composition have been debated, and could be potentially identified by observing the common core and bacterial communities associated with alternate stable states. The core phylotypes have co-evolved to provide crucial ecosystem services such as production of butyrate (energy source for colonic epithelial cells) and protection against invasive pathogens and outgrowth of endogenous pathobionts. The process of modulating microbiome can be initiated in early life by managing certain host associated and environmental factors like diet. The mode of delivery has an effect on the pattern of development of the intestinal bacterial community (Grölund *et al.* 1999; Cox *et al.* 2014). Caesarean delivery is associated with a reduced number of *Clostridium* species most of which are part of the core microbiota in adult life (Fig. 1) (Bokulich *et al.* 2016). However, another recent report did not observe associations between the delivery mode and adult microbiota composition (Falony *et al.* 2016). Maternal health status (obesity) and maternal stress have also been associated with an aberrant intestinal bacterial community in infants (Galley *et al.* 2014; Zijlmans *et al.* 2015). However, modifying the dietary habits, breast feeding and incorporating specific oligosaccharides have shown to aid in improving the abundance of anaerobic bacteria (Haarman and Knol 2006). Exercising regularly is known to have various health benefits. A recent study on professional athletes revealed that exercise could aid in increasing intestinal bacterial diversity (Clarke *et al.* 2014). In elderly subjects, the intestinal microbiome is characterized by reduced bacterial diversity (Biagi *et al.* 2010; Claesson *et al.* 2012). Reduced abundance of beneficial core bacteria, increased inflammation and a decrease in the concentration of SCFA's have also been observed in elderly subjects (Salazar *et al.* 2013). These deviations

from a normal adult healthy state could be reduced by targeted modulation, which may include restoring the healthy core phylotypes (through probiotic and/or prebiotic interventions), reducing proinflammatory bacteria and increasing the SCFA production by incorporating dietary fibers in the daily diet.

CONCLUDING REMARKS

In the past few decades, we have gained significant insights into the composition, dynamics and function of our intestinal microbiome and its impact on our well-being. The Western adult population shares dozens of genus-level taxonomic groups that show high functional correlation. The intestinal microbiome should be viewed as a complex landscape, which is a mixture of common and individual characteristics. In addition to continuously changing gradients across bacterial abundance and function, this intestinal microbiome landscape also includes distinct features present as alternative stable states across varied compositional, structural and functional features. It is now clear that treatment for most of the disease conditions necessities careful consideration of the intestinal microbiome. A recent example is the application of a prediction algorithm based on dietary habits, physical activity and gut microbiota that allowed predicting blood glucose levels and designing personalized diets which were effective in controlling post-prandial blood glucose levels (Zeevi *et al.* 2015). While the field of microbiome research rapidly moves forward, there has to be a careful consideration of the confounding variables in the studies, knowing that correlation does not imply causation, technical bias between studies, differences in statistical models, human error and most importantly reproducibility (Ioannidis 2005; Falony *et al.* 2016). As the microbiome research moves into the next phase of diagnostics and therapeutics, overcoming these issues will be a major focus of future research.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSRE* online.

AUTHOR CONTRIBUTIONS

SAS designed and coordinated the manuscript preparation, and took main responsibility on the writing. LL assisted in generation of figures. FH, LL, HS and WMdV provided critical reviews and suggestions for the content, and contributed to the text. All authors read and approved the final manuscript.

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