Original Article

Gut microbiome is more stable in males than in females during the development of colorectal cancer

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Abstract

Aims: Gut microbial alterations have great potential to predict the development of colorectal cancer (CRC); however, how gut microbes respond to the development of CRC in males and females at the community level is unknown. We aim to investigate the differences of gut microbiota between the male and female.

Methods and Results: We reanalysed the dataset in a published project from a sex perspective at the community level by characterizing the gut microbiome in patients (including males and females) from three clinical groups representative of the stages of CRC development: healthy, adenoma, and carcinoma. The results indicated that the microbial α-diversity showed no significant difference in the male gut but had decreased significantly in the female gut with the development of CRC. In males, a significant difference in the microbial β-diversity was only observed between the healthy and carcinoma subgroups. However, significant community deviations were detected with the development of CRC in females. The microbial community assembly processes changed from deterministic to stochastic in males, whereas they became increasingly deterministic in females with the development of CRC. Moreover microbial co-occurrence associations tended to be more complicated in males; rare species were enriched in the co-occurrence network of the male gut, whereas key species loss was observed in the co-occurrence network of the female gut.

Conclusions: The microbial communities in the male gut were more stable than those in the female gut, and microbial community assembly in the gut was sex dependent with the development of CRC. Our study suggests that sexual dimorphism needs to be considered to better predict the risk of CRC based on microbial shifts.

Significance and Impact of the Study: To the best of our knowledge, this is the first report showing how gut microbes respond to the development of CRC in males and females at the community scale.

Introduction

Colorectal cancer (CRC) is highly associated with inflammatory bowel disease (Lasry et al. 2016), and dysbiosis of gut microbes has been recognized as one of the drivers of inflammatory bowel disease (Tamboli et al. 2004) that acts by altering the interactions between microbes and the mucosal immune system (Dalal and Chang 2014; Kaiko and Stappenbeck 2014). Previous studies have demonstrated that the microbial communities in CRC patients deviate from those in normal individuals (Uemura et al. 2001; Thomas et al. 2016; Flemer et al.)
2017), therefore, microbial communities are likely altered with the development of CRC. However, the majority of studies have focus on CRC risk detection research with biomarkers and prediction models of gut microbes at taxon level (Zackular et al. 2014; Villeged et al. 2018; Wang et al. 2019; Yang et al. 2019), it is little known about the unique gut microbes at community level in CRC patients. Recently, the bioinformatics analysis, such network analysis and decision tree aggregation with a random forest model, and metagenomic and metabolomic analyses have been applied to identify microbial diagnostic signatures in CRC datasets (Wirbel et al. 2019; Yachida et al. 2019; Ai et al. 2019a,b). This might offer us a new understanding on the relationship between microbial communities of gut and the development of CRC.

Gut microbial changes and disease are highly associated with sex differences (Elin et al. 2016; Fransen et al. 2017). For example, gut microbial communities in males differ from those in females in the face of external environmental pressures (Wang et al. 2016; Takagi et al. 2019). Moreover studies focusing on abdominal obesity-related disease (Min et al. 2019), type I diabetes (Markle et al. 2013; Yurkovetskiy et al. 2013) and major depressive disorder (Chen et al. 2018) have also demonstrated that sex-specific differences are important for shaping the gut microbial community with the development of related diseases. This may be because sexual maturation, hormones, and differences in the immune system and function profoundly affect microbe colonization in the gut (Markle et al. 2013; Elin et al. 2016; Fransen et al. 2017). However, it is unclear whether the microbial aggregates in gut are sex dependent and whether the mechanisms of species assembly are correlated with sex with the development of CRC.

Community assemblies describe microbial responses to environmental changes at the community scale and have been successfully applied in the microbial ecology of natural ecosystems (Tripathi et al. 2018). Microbial community assembly is associated with both deterministic and stochastic processes (Zhou et al. 2014). Deterministic processes are involved in the ecological selection of both biotic and abiotic factors, which influence microbial assembly by changing the fitness between organisms and the environment and therefore eventually altering community composition and species abundance (Wang et al. 2013). Stochastic processes include dispersal, random birth, death and ecological drifts, which result in communities that are similar to those produced by chance (Kraft et al. 2011). The balance between these two ecological processes could reflect how microbes respond to the environment at the community scale.
Materials and methods

Data collection
All 90 faecal samples in the present study were derived from a published project (Zackular et al. 2014). Raw sequence reads are available at http://www.mothur.org/ MicrobiomeBiomarkerCRC. Briefly, faecal samples were randomly collected from healthy participants \((n = 30)\), colonic adenoma \((n = 30)\) and colonic adenocarcinoma patients \((n = 30)\) between 1 and 4 weeks after colonscopy preparation. The 16S rRNA genes in each sample were amplified and sequenced using the Illumina MiSeq platform. To compare the differences between male and female individuals, we classified the 90 samples into two groups: male \((n = 50)\); contained healthy \((n = 11)\), colorectal adenoma \((n = 18)\) and carcinoma \((n = 21)\) subgroups and female \((n = 40)\); contained healthy \((n = 19)\), colorectal adenoma \((n = 12)\) and carcinoma \((n = 9)\) subgroups.

Bioinformatics analysis
Raw paired-end reads were assembled and filtered using USEARCH 8.1 (Edgar 2010; Edgar et al. 2011). Sequences with ambiguous nucleotides, lengths <200 bps or expected error values >1 were discarded. Chimeric sequences in our dataset were removed using the UCHIME algorithm, and clean data were clustered into operational taxonomic units (OTUs) at a 3% cutoff with the UPARSE algorithm. OTU representatives were assigned using the SILVA 16S rRNA gene database (Pruesse et al. 2007). OTUs that were assigned to mitochondrial DNA were removed before further analysis. We therefore resampled 25 747 sequences for each sample to ensure the accuracy of the group across comparisons and obtained 8220 OTUs with 9 046 493 sequences in total after removal of singletons. The \(x\)-diversity indices were calculated using QIIME 1.9.

Ecological statistical analysis
Gut microbial communities were visualized using principal coordinate analysis (PCoA) with Bray–Curtis dissimilarities, and the overall differences in microbial community composition among developmental stages of CRC were tested using analysis of similarity (ANOSIM). The ANOSIM was performed in R vegan package (see Supporting Information). The Bray–Curtis dissimilarity is a statistic used to quantify the compositional dissimilarity between two different sites, based on counts at each site (Bray and Curtis 1957; Zhao et al. 2015). It is bounded between 0 and 1, where 0 means that the two sites have the same composition (means they share all the species), and 1 means that the two sites do not share any species (Kaesbohrer et al. 2019). The Bray–Curtis dissimilarity index (BC) was calculated by function ‘vegdist(‘) in R vegan package (Oksanen et al. 2017). Microbial \(x\)-diversities were compared using a random permutation test (RPT) with 999 iterations. The RPT is a statistical method used to test significance between two groups, and the details of this method are listed in the Supporting Information. To estimate potential metabolic changes in microbial communities during the development of CRC, potential functional genes were predicted using the Tax4Fun R package (Asshauer et al. 2015). Unweighted Venn diagrams were further applied to compare shared and specific OTUs/functional genes among CRC development stages.

Null model analysis
Underlying microbial assembly patterns were estimated using null models (Chase et al. 2011; Zhou et al. 2014). The modified Raup–Crick dissimilarity metric was used to calculate microbial community dispersion for the CRC proceeding stage in males and females, which is robust to variations in local species richness (Chase et al. 2011). This metric is an indicator of compositional differences based on species presence–absence data between the observed communities relative to those generated under the null model that is used by estimating the probability that any two null communities drawn randomly from the “regional” species pool have the same number or more species in common than the observed communities (Chase et al. 2011). This modified Raup–Crick dissimilarity metric measured the deviation in community dissimilarities from the null distribution under random assembling, which allowed us to infer the relative dominance of different community assemblage processes in males and females during the development of CRC (Chase et al. 2011). A high community dispersion implied the high importance of stochastic processes in microbial community assembly; otherwise, deterministic processes might dominate (Zhou et al. 2014). This analysis was performed by the method that provided by Chase et al (2011) and the null expectation was calculated using 9999 randomizations using the ‘raupcrick()‘ function in the vegan package for R (Valverde et al. 2014). Moreover the modified Raup–Crick dissimilarity metric was visualized by non-metric multidimensional scaling analyses (NMDS), which was performed with the ‘metaMDS(‘) function in vegan package. The larger circles mean more stochastic of the community assembly, whereas small circles mean more deterministic in the NMDS. Differences in microbial community dispersion among the
developmental stages of CRC were tested using permutational analysis of multivariate dispersion (PERMDISP, 999 iterations).

The observed and expected Jaccard dissimilarities based on random resampling were further calculated for each developmental stage of CRC with 999 iterations. The Jaccard dissimilarity (1-similarity) is a metric used for comparing the dissimilarity and diversity of sample sets (Jaiganesh and Jaganathan 2015), which was calculated by function ‘vegdist()’ in R vegan package. Of particular note is that the sample counts in male and female individuals are distinct. This may introduce some bias to our final results because of differences in the size of species pool. Therefore, the β-deviation, which was defined as the divergence between observed and mean expected matrices divided by the standard deviation of expected values (standard effect size, SES) and is usually used to measure the real β-diversity after controlling random sampling effects, was calculated (see Supporting Information). Differences between observed and expected β-diversity as well as the β-deviation divergences among CRC stages were tested using PERMDISP with 999 iterations. The species pools in both Raup-Crick and Jaccard’s dissimilarity-based null models were defined as the total species number in each developmental stage of each sex.

Co-occurrence network analysis

To evaluate the response of microbial interactions to CRC, we inferred two meta co-occurrence networks for male and female gut microbial communities. Briefly, the Spearman rank correlation coefficients among OTUs were first calculated, and P-values were adjusted using the Benjamini and Hochberg false discovery rate (Benjamini et al. 2006). A random matrix theory-based approach (Luo et al. 2006) was used to determine the correlation cutoffs. Co-occurrence networks without self-connections were inferred using igraph (https://igraph.org/) python package (male: ρ = 0.70, P < 0.001; female: ρ = 0.73, P < 0.001). The subnetworks of each sample and developmental stage of CRC were split from the meta network by preserving OTUs presented in each sample and the group (Ma et al. 2016). The nodes in these networks represent OTUs, and the edges that connect these nodes represent correlations between OTUs.

Node-level topological properties encompassing betweenness centrality (the number of shortest paths going through a node) and degree centrality (the number of edges that connect to a node) were calculated for subnetworks. The betweenness centrality feature was used to measure the centrality of each node in the subnetwork. Nodes in each subnetwork were further classified as peripheral, intermediate or central by ranking all nodes according to betweenness centrality and partitioning this ranked list into three equally populated bins, which were termed ‘centrality tiers’ (Ma et al. 2016). Network-level topological properties, including the node count, edge count, average degree, average path length, clustering coefficient, cluster number, modularity, diameter, degree assortativity and density, were further calculated for meta-and subnetworks. Detailed definitions of these topological properties were described in Supporting Information Table S1. Topological structures of subnetworks from each developmental stage of CRC were compared using principal component analysis (PCA), and ANOSIM based on network-level topological indices was calculated. All topological properties were calculated using igraph (https://igraph.org/) python package, and network modules were detected with a greedy modularity optimization algorithm. Differences in subnetwork properties among developmental stages of CRC were tested using a random permutation test with 999 iterations. Network images were generated using Gephi (http://gephi.github.io/).

Results

Landscape of the microbial community

The microbial α-diversities were not significantly different during the development of CRC in the male gut (P > 0.05), while they were significantly decreased during the development of CRC in the female gut (P < 0.05) (Fig. 1a). Moreover a significant difference in microbial composition was only observed between the healthy and carcinoma subgroups in males (R = 0.063, P < 0.05) (Fig. 1b). However, significant community deviations were detected with the development of CRC in females (Fig. 1c). Shared OTU counts during the developmental stages of CRC in males were higher than those in females (Fig. 1d,e). Specific OTU counts increased with the development of CRC in males (Fig. 1d) but decreased in females (Fig. 1e). Shared functional gene counts were similar in males and females (Fig. 1f,g). Although functional gene counts decreased with the development of CRC in both males and females, changes in the female gut were greater than those in the male gut (Fig. 1f,g). In addition, species loss occurred in both males and females with the development of CRC (Figs S1 and S2).

Microbial community assembly

Microbial community dispersion increased in males (Fig. 2a) but decreased in females (Fig. 2b) with the development of CRC. The observed microbial β-diversities in the male gut were significantly different from the null distribution in the healthy and adenoma subgroups.
(P < 0.001), but there was no significant difference in carcinoma patients (P = 0.088) (Fig. 2c). However, the observed microbial β-diversities in the female gut did not have significant deviations from the null distribution (P > 0.05) (Fig. 2c). The observed β-diversities increased with the development of CRC in males but gradually decreased in females (Fig. 2d). These results indicated that the microbial community assembly processes change from deterministic to stochastic in males with the development of CRC. Although the microbial community assembly exhibited stochastic processes in females, it also showed increasing deterministic processes with the development of CRC. Values of the SES among the development stages of CRC showed no apparent changes compared to the observed β-diversity in males (except between the healthy and carcinoma subgroups) (Fig. 2d). In contrast, the SES in females dramatically decreased with the development of CRC (Fig. 2d).

**Microbial co-occurrence pattern**

The microbial meta co-occurrence network in the male gut included 5689 nodes and 90,760 edges, while the meta co-occurrence network in the female gut contained 77,167 associations among 5522 nodes (Fig. 3, Supporting Information Table S2). Nodes in the male meta co-
occurrence network were highly aggregated, but they tended to cluster into network modules in the female meta co-occurrence network (Fig. 3, Supporting Information Table S2). Connections among network modules in the male meta co-occurrence network were stronger than those in the female meta co-occurrence network (Fig. 3). *Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria* were detected with high abundance (>1%) in both meta co-occurrence networks, but *Tenericutes* (>1%) was only observed in the female meta co-occurrence network (Fig. 3).

With the development of CRC, links among network modules increased in the male subnetworks but decreased in the female subnetworks (Fig. 4). The node degree and betweenness centralities increased in the male subnetworks but decreased in the female subnetworks with the
development of CRC (Fig. 5). Centrality-based tiers indicated a distinct response of core network nodes to CRC between males and females (Fig. 6). Microbes belonging to the Parcubacteria phylum were detected in the intermediate tier of the male adenoma network. Although Parcubacteria disappeared, Spirochaetes, Lentisphaerae and Planctomycetes arose in the male cancer network. The Synergistetes phylum was primarily located at the centre of the female healthy network, but some of the members of this phylum migrated to the intermediate tier with CRC. Lentisphaerae and Acidobacteria were detected in the intermediate tier of the healthy female network, but they were excluded from the adenoma and cancer networks (Fig. 6).

Principal component analysis (PCA) of network topological properties revealed that network structures had little correlation with CRC in males (Fig. S3a). However, the CRC deviated significantly from network structures in females (Fig. S3b). Topological properties including the node count, edge count, average path length, cluster number, modularity and diameter were significantly decreased, whereas the network density was increased in CRC patients in the female networks (Fig. S4). In contrast, all topological properties were detected without significant changes in male networks during the development of CRC (Fig. S4).

**Discussion**

The human gut microbiome has been recognized to be correlated with the development of CRC and has great potential to predict the risk and clinical status of CRC (Zackular et al. 2014; Zeller et al. 2014; Sze and
Schloss 2018; Villeger et al. 2018). Previous study has indicated that the microbial structure of the human gut microbiome is sex dependent (Takagi et al. 2019). However, it is unclear whether the ecological responses of gut microbial communities to CRC development were sex dependent. In this paper, to answer this question, we reanalysed 90 faecal samples from the dataset of Zackular et al. (2014). The results revealed that the responses of gut microbial communities to CRC are sex dependent. Furthermore, the enrichment of rare species may contribute to the stability of microbial communities in the male gut, whereas species loss may be responsible for the vulnerable microbial communities in the female gut with the development of CRC. Therefore, sex needs to be considered to accurately predict the risk of CRC.

Changes in community structure in the male and female gut

This study showed that species depletion in females (Fig. S2) may induce \( \alpha \) - diversities and community dispersions that drastically decrease with the development of CRC. However, although species losses were also observed in males (Fig. S1), the \( \alpha \) - diversities were not significantly different, and increased community dispersion occurred during the development of CRC. These results indicated that microbial diversity is more susceptible to disturbances by CRC in female gut. Our results are consistent with the findings of Huang et al. (2018) regarding the sex-dependent changes in gut microbial communities during the development of hepatocellular carcinoma in mice; their findings demonstrated that the microbial community in females is more easily disordered than that in males. Differences in the community structure may be explained by the different responses of hormonal factors and immune system to CRC between males and females (Overbeek et al. 2019; Takagi et al. 2019). Collectively, our results indicated that the relationships between CRC and the gut microbial structure are sex dependent. Moreover we speculated that the divergence in the \( \alpha \) - and \( \beta \) - diversities might imply a difference in the community assembly pattern in the male and female gut with the development of CRC.

Figure 4 Microbial co-occurrence network variations with the development of colorectal carcinoma in the male and female gut. The nodes in the network represent OTUs in the community, and the edges represent the correlations among OTUs. All nodes were colored based on network modules (only modules with nodes with an abundance greater >1% in the network are colored); those isolated nodes or nodes with extremely low degree centrality may not be seen in these two networks. [Colour figure can be viewed at wileyonlinelibrary.com]
Different community assembly patterns in the male and female gut

The microbial community assembly pattern revealed how microbes responded to the development of CRC at the community scale. In our study, the microbial community assembly processes changed from deterministic to stochastic in males, whereas they became increasingly deterministic in females with the development of CRC. Previous studies have demonstrated that deterministic processes governed microbial community assembly in both healthy and diseased individuals, and host immunity largely determines this process (Jeraldo et al. 2012; Dai et al. 2017; Sun et al. 2019). A recent study indicated that sexual divergence in the immune system largely contributes to microbial colonization in the gut, which in turn further governs the sexual discrepancies in the immune system (Fransen et al. 2017). Combined with our results, this suggests that the

Figure 5 Changes in the degree and betweenness centrality of microbial co-occurrence networks during the development of colorectal carcinoma in the male (a) and female gut (b). The significance tests were carried out using a random permutation test (RPT). \( *P < 0.05, **P < 0.01, ***P < 0.001 \). [Colour figure can be viewed at wileyonlinelibrary.com]
patterns of microbial community assemblies are sex dependent during the development of CRC. This may be caused by the high filtering pressures on the gut microbial communities in females compared with males (Li et al. 2019). Specifically, although the size of the species pool increased, both the α-diversity and community composition (β-diversity) were not changed or were less changed in the male gut than in the female gut with the development of CRC (Fig. 1a,c), suggesting that rare species in individuals with CRC govern the change in the microbial community typical of healthy individuals. This is because vast rare species in communities results in a high probability of some ecological events (e.g. ecological drifts associated with random birth and death, replacement of individuals) (Burns et al. 2016) and further increases the importance of stochastic processes in the gut of males with CRC. In contrast, species loss with the development of CRC in the female gut might deterministically alter the microbial community structure and eventually increase the importance of deterministic processes, which is supported by the fact that the species pool size and α- and β-diversities decreased with the development of CRC. The results from our present study imply that the vulnerability of female gut microbial communities to CRC primarily results from species loss. Moreover our study suggests that the decreased diversity and more deterministic processes in females and the increased diversity and more stochastic processes in males might indicate an increasing risk of CRC.

Changes in the co-occurrence networks in the male and female gut

The co-occurrence networks inferred from the microbial communities in males showed highly complex relationships among microbial taxa compared with those in females, indicating higher microbial community stability.

Figure 6 Percentage of nodes belonging to different phyla in different centrality tiers. These relative positions of each node in the network were inferred using betweenness centrality. The nodes with high betweenness centrality are closer to the center of the network compared to those with low betweenness centrality, and vice versa. [Colour figure can be viewed at wileyonlinelibrary.com]
in the male gut (Fan et al. 2018). Indeed, associations among network modules in male subnetworks were increased; however, they were decreased significantly in female subnetworks with the development of CRC (Fig. 4), as well as the variation in the topological properties (such as average degree) of gut microbial networks in males and females (Fig. S4). In addition, centrality-tie-based analysis showed that rare species enriched in male gut microbial communities might serve as key hubs that maintain microbial community stability during the development of CRC and that ecological compensatory effects likely improve the inner- or interspecies interactions in male gut microbial communities to adapt to physiological changes in the host gut (Greenblum et al. 2012). However, depleted species might have important functions and serve as the key nodes in the subnetworks in the gut microbial communities of females during the development of CRC. Furthermore, we found that the Parcubacteria phylum (also known as candidate phylum OD1) was located in the intermediate tier of male subnetworks when colorectal adenoma occurred (Fig. 6). According to previous studies, microbes belonging to the Parcubacteria phylum are ectosymbionts or parasites (Nelson and Stegen 2015). Although the putative role of these highly adapted organisms in CRC remains poorly understood (Thomas et al. 2016), it can be suggested that Parcubacteria might largely contribute to the stability of the microbial network at the colorectal adenoma stage in males. In addition, Lentisphaerae, Planctomycetes and Spirochaetes (located in the central and intermediate tiers) were only detected in males with CRC, showing potential as biomarkers for CRC in males. Recent studies have shown that microbes belonging to the Lentisphaerae and Planctomycetes phyla possibly played roles in carbon recycling and dissolved organic production and acted as additional sources of energy and carbon in the natural environment (such as water) (Yilmaz et al. 2015; Spring et al. 2016), suggesting that these microbes might possess high adaptability to shifts in the environment and play vital roles in maintaining stability in males with the development of CRC. Unlike the variation in phyla in the male gut, decreased abundance of the phyla Lentisphaerae and Acidobacteria was observed in the female gut with the development of CRC, suggesting that these phyla might contribute to the weakening of the network in females. These results indicated that both the increased abundance of Lentisphaerae, Planctomycetes and Spirochaetes in males and the decreased abundance of Lentisphaerae and Acidobacteria in females might increase the risk of CRC.

Furthermore, we proposed two hypotheses, namely, ‘role effects’ and ‘compensatory effects’, to explain how the microbial community responds to CRC in males and females. The ‘role effects’ occur when there is key species loss, reducing the core functionality. The ‘compensatory effects’ occur when subdominant species take over functionality from the ones that are lost (Ulrich et al. 2019).

In our study, the ‘compensatory effects’ dominated in males, whereas ‘role effects’ played more important roles in females with the development of CRC. These findings were supported by the increasing functional genes, higher node counts and stronger connections among network modules in males than in females with the development of CRC. This phenomenon could be explained in two ways. First, the species lost with the development of CRC might exhibit lower abundance, competition and growth rates (Logares et al. 2015) or contribute less to microbial functions in males. However, depleted species likely have higher abundance and govern most ecological functions in females. Second, CRC might trigger species replacement in both the male and female gut, and these newly enriched species likely compensated for most ecological functions of depleted species in the male gut. However, the important functionalities performed by the lost high-abundance microbes cannot be compensated for by the replacement species in females. Therefore, we may infer that compensatory effects are more important than role effects in males, whereas role effects play major roles in females in regard to the development of CRC.

In summary, we demonstrated that the gut microbial community responds distinctly to the development of CRC in males and females. Our findings suggest that microbial communities are more stable in the male gut than in the female gut during the development of CRC. Furthermore, the females likely sustain high risks of shifts in the microbiome, including changes in diversity, community structure, and microbial interactions. From the null model analysis, the gut microbes of females became more deterministic, and the gut microbes of males became more stochastic, which might indicate an increasing risk of CRC. These results combined with other existing indicators can enhance the accuracy of the prediction of CRC. Collectively, the findings of this study highlight an important role of sex in the accurate prediction of the risk of CRC.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

**References**

Ai, D., Pan, H., Han, R., Li, X., Liu, G. and Xia, L.C. (2019a)
Using decision tree aggregation with random forest model to identify gut microbes associated with colorectal cancer.
*Genes (Basel)* 10, 112.

Ai, D., Pan, H., Li, X., Wu, M. and Xia, L.C. (2019b)
Association network analysis identifies enzymatic components of gut microbiota that significantly differ between colorectal cancer patients and healthy controls.
*PeerJ* 7, e7315.

Asshauer, K.P., Wemheuer, B., Daniel, R. and Meinicke, P. (2019)
Michigan or Department of Family Medicine, University of Michigan.

References

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. and Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection.
*Bioinformatics* 27, 2194–2200.

Elin, O., Margarete, M., Parks, B.W., Petia, S., Liu, X., Drake, T.A. and Lusis, A.J. (2016) Sex differences and hormonal effects on gut microbiota composition in mice.
*Gut Microbes* 7, 313–322.

Fan, K., Weisenhorn, P., Gilbert, J.A. and Chu, H. (2018)
Wheat rhizosphere harbors a less complex and more stable microbial co-occurrence pattern than bulk soil.
*Soil Biol Biochem* 125, 251–260.

Faust, K., Sathirapongsasuti, J.F., Izard, J., Segata, N., Gevers, D., Raes, J. and Huttenhower, C. (2012) Microbial co-occurrence relationships in the human microbiome.
*PLoS Comp Biol* 8, e1002606.

Feng, M., Adams, J.M., Fan, K., Shi, Y., Sun, R., Wang, D., Guo, X. and Chu, H. (2018) Long-term fertilization influences community assembly processes of soil diazotrophs.
*Soil Biol Biochem* 126, 151–158.

Flemmer, B., Lynch, D.B., Brown, J.M.R., Jeffery, I.B., Ryan, F.J., Claesson, M.J., O’Riordain, M., Shanahan, F. et al. (2017) Tumour-associated and non-tumour-associated microbiota in colorectal cancer.
*Gut* 66, 633–643.

Fransen, F., Beek, A.A.V., Borghuis, T., Meijer, B., Hugenholtz, F., Jongs, C.V.D.G., Savellkouil, H.F., Jonge, M.I.D. et al. (2017) The impact of gut microbiota on gender-specific differences in immunity.
*Front Immunol* 8, 754.

Greenblum, S., Turnbaugh, P.J. and Borenstein, E. (2012) Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease.
*Proc Natl Acad Sci USA* 109, 594–599.

Huang, R., Li, T., Ni, J., Bai, X., Gao, Y., Li, Y., Zhang, P. and Gong, Y. (2018) Different sex-based responses of gut microbiota during the development of hepatocellular carcinoma in liver-specific Tsc1-knockout mice.
*Front Microbiol* 9, 1008.

Jaiaganesh, S. and Jaganathan, P. (2015). An appropriate similarity measure for K-means algorithm in clustering web documents. *IJSRD* 3.

Jeraldo, P., Sipos, M., Chia, N., Bruck, J.M., Dhillon, A.S., Konkel, M.E., Larson, C.L., Nelson, K.E. et al. (2012) Quantification of the relative roles of niche and neutral processes in structuring gastrointestinal microbiomes.
*Proc Natl Acad Sci USA* 109, 9692–9698.

Kaesbohrer, A., Bakran-Lehl, K., Irgang, A., Fischer, J., Kämpf, P., Schiiffmann, A., Werckenthin, C., Busch, M. et al. (2019) Diversity in prevalence and characteristics of ESBL/AmpC producing *E. coli* in food in Germany.
*Vet Microbiol* 233, 52–60.

Kaiko, G.E. and Stappenbeck, T.S. (2014) Host-microbe interactions shaping the gastrointestinal environment.
*Trends Immunol* 35, 538–548.

Kang, M. and Martin, A. (2017) Microbiome and colorectal cancer: unraveling host-microbiota interactions in colitis-
associated colorectal cancer development. *Semin Immunol* 32, 3–13.

Kara, E.L., Hanson, P.C., Hu, Y.H., Winslow, L. and McMahon, K.D. (2013) A decade of seasonal dynamics and co-occurrences within freshwater bacterioplankton communities from eutrophic Lake Mendota, WI, USA. *ISME J* 7, 680–684.

Kraft, N.J., Comita, L.S., Chase, J.M., Sanders, N.J., Swenson, N.G., Crist, T.O., Stegen, J.C., Vellend, M. et al. (2011) Disentangling the drivers of beta diversity along latitudinal and elevational gradients. *Science* 333, 1755–1758.

Lasry, A., Zinger, A. and Ben-Neriah, Y. (2016) Inflammatory networks underlying colorectal cancer. *Nat Immunol* 17, 230.

Li, H., Zhou, R., Zhu, J., Huang, X. and Qu, J. (2019) Environmental filtering increases with elevation for the assembly of gut microbiota in wild pikas. *Microb Biotechnol* 12, 976–992.

Llamas, M.E., Huber, P., Metz, S. and Unrein, F. (2017) Interplay between stochastic and deterministic processes in the maintenance of alternative community states in Verrucomicrobia-dominated shallow lakes. *FEMS Microbiol Ecol* 93, fbx077.

Logares, R., Mangot, J.F. and Massana, R. (2015) Rarity in aquatic microbes: placing protists on the map. *Microbiol Ecol* 66, 831–841.

Luo, F., Zhong, J., Yang, Y., Scheuermann, R.H. and Zhou, J. (2006) Application of random matrix theory to biological networks. *Phys Lett A* 357, 420–423.

Ma, B., Wang, H., Dsouza, M., Lou, J., He, Y., Dai, Z., Brookes, P.C., Xu, J. et al. (2016) Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *ISME J* 10, 1891–1901.

Markle, J.G., Frank, D.N., Mortin-Toth, S., Robertson, C.E., Feazel, L.M., Rolle-Kampczyk, U., Von Bergen, M., McCoy, K.D. et al. (2013) Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 339, 1084–1088.

Min, Y., Ma, X., Sankaran, K., Ru, Y., Chen, L., Baiocchi, M. and Zhu, S. (2019) Sex-specific association between gut microbiome and fat distribution. *Nat Commun* 10, 2408.

Nelson, W.C. and Stegen, J.C. (2015) The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. *Front Microbiol* 6, 713.

Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O’Hara, R. et al. (2017). Vegan: community ecology package. 2017. R package version 2.4–4. https://CRAN.R-project.org/package=vegan.

Overbeek, J.A., Kuiper, J.G., van der Heijden, A.A.W.A., Labots, M., Haug, U., Herings, R.M.C. and Nijpels, G. (2019) Sex- and site-specific differences in colorectal cancer risk among people with type 2 diabetes. *Int J Colorectal Dis* 34, 269–276.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplis, J. and Glockner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* 35, 7188–7196.

Spring, S., Bunk, B., Sproer, C., Schumann, P., Rohde, M., Tindall, B.J. and Klenk, H.P. (2016) Characterization of the first cultured representative of Verrucomicrobia subdivision 5 indicates the proposal of a novel phylum. *ISME J* 10, 2801–2816.

Steele, J.A., Countway, P.D., Xia, L., Vigil, P.D., Beman, J.M., Kim, D.Y., Chow, C.E., Sachdeva, R. et al. (2011) Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *ISME J* 5, 1414–1425.

Sun, Y., Li, L., Xia, Y., Li, W., Wang, K., Wang, L., Miao, Y. and Ma, S. (2019) The gut microbiota heterogeneity and assembly changes associated with the IBD. *Sci Rep* 9, 440.

Sze, M.A. and Schloss, P.D. (2018) Leveraging existing 16S rRNA gene surveys to identify reproducible biomarkers in individuals with colorectal tumors. *MBio* 9, https://doi.org/10.1128/mBio.00630-18.

Takagi, T., Naito, Y., Inoue, R., Kashiwagi, S., Uchiyama, K., Mizushima, K., Tsuchiya, S., Dohi, O. et al. (2019) Differences in gut microbiota associated with age, sex, and stool consistency in healthy Japanese subjects. *J Gastroenterol* 54, 53–63.

Tamboli, C.P., Neut, C., Desreumaux, P. and Colombel, J.F. (2004) Dysbiosis in inflammatory bowel disease. *Gut* 53, 1–4.

Thomas, A.M., Jesus, E.C., Lopes, A., Samuel Aguilar, J., Begnami, M.D., Rocha, R.M., Carpinetti, P.A., Camargo, A.A. et al. (2016) Tissue-associated bacterial alterations in rectal carcinoma patients revealed by 16S rRNA community profiling. *Front Cell Infect Microbiol* 6, 179.

Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M. and Lee, Y.K. (2018) Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. *ISME J* 12, 1072–1083.

Uemura, N., Okamoto, S., Yamamoto, S., Matsumura, N., Yamaguchi, S., Yamakido, M., Taniyama, K., Sasaki, N. et al. (2001) *Helicobacter pylori* infection and the development of gastric cancer. *New Engl J Med* 345, 784–789.

Ulrich, W., Hulisz, P., Mantilla-Contreras, J., Elvisto, T. and Piernik, A. (2019) Compensatory effects stabilize the functioning of Baltic brackish and salt marsh plant communities. *Estuar Coast Shelf Sci* 231, https://doi.org/10.1016/j.ecss.2019.106480.

Valverde, A., Makhalanyane, T.P. and Cowan, D.A. (2014) Contrasting assembly processes in a bacterial metacommunity along a desiccation gradient. *Front Microbiol* 5, 668.

Villeger, R., Lopes, A., Veziant, J., Gagniere, J., Barnich, N., Billard, E., Boucher, D. and Bonnet, M. (2018) Microbial markers in colorectal cancer detection and/or prognosis. *World J Gastroenterol* 24, 2327–2347.

Wang, G., Yu, Y., Wang, Y.Z., Wang, J.J., Guan, R., Sun, Y., Shi, F., Gao, J., Li, J., Zhao, Y., Xu, J. et al. (2019) Role of SCFAs in gut
microbiome and glycolysis for colorectal cancer therapy. J Cell Physiol 234, 17023–17049.
Wang, J., Shen, J., Wu, Y., Tu, C., Soininen, J., Stegen, J.C., He, J., Liu, X. et al. (2013) Phylogenetic beta diversity in bacterial assemblages across ecosystems: deterministic versus stochastic processes. ISME J 7, 1310–1321.
Wang, J., Wang, J., Pang, X., Zhao, L., Tian, L. and Wang, X. (2016) Sex differences in colonization of gut microbiota from a man with short-term vegetarian and inulin-supplemented diet in germ-free mice. Sci Rep 6, 36137.
Wirbel, J., Pyl, P.T., Kartal, E., Zych, K., Kashani, A., Milanese, A., Fleck, J.S., Voigt, A.Y. et al. (2019) Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. Nat Med 25, 679–689.
Yachida, S., Mizutani, S., Shiroma, H., Shiba, S., Nakajima, T., Sakamoto, T., Watanabe, H., Masuda, K. et al. (2019) Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. Nat Med 25, 968–976.
Yang, J., McDowell, A., Kim, E.K., Seo, H., Lee, W.H., Moon, C.M., Kym, S.M., Lee, D.H. et al. (2019) Development of a colorectal cancer diagnostic model and dietary risk assessment through gut microbiome analysis. Exp Mol Med 51, 1–15.
Yilmaz, P., Yarza, P., Rapp, J.Z. and Glockner, F.O. (2015) Expanding the world of marine bacterial and archaeal clades. Front Microbiol 6, 1524.
Yurkovetskiy, L., Burrows, M., Khan, A.A., Graham, L., Volchkov, P., Becker, L., Antonopoulos, D., Umesaki, Y. et al. (2013) Gender bias in autoimmunity is influenced by microbiota. Immunity 39, 400–412.
Zackular, J.P., Rogers, M.A.M., Ruffin, M.T. and Schloss, P.D. (2014) The human gut microbiome as a screening tool for colorectal cancer. Cancer Prev Res (Phila) 7, 1112–1121.
Zeller, G., Tap, J., Voigt, A.Y., Sunagawa, S., Kultima, J.R., Costea, P.I., Amiot, A., Bohm, J. et al. (2014) Potential of fecal microbiota for early-stage detection of colorectal cancer. Mol Syst Biol 10, 766.
Zhao, N., Chen, J., Carroll, I.M., Ringel-Kulka, T., Epstein, M.P., Zhou, H., Zhou, J.J., Ringel, Y. et al. (2015) Testing in microbiome-profiling studies with MiRKAT, the microbiome regression-based kernel association test. Am J Hum Genet 96, 797–807.
Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D., Yang, Y., He, Z. et al. (2014) Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. Proc Natl Acad Sci USA 111, E836–E845.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Appearance/disappearance of all observed OTUs with the development of colorectal carcinoma in the gut microbial communities of males.
Figure S2. Appearance/disappearance of all observed OTUs with the development of colorectal carcinoma in the gut microbial communities of females.
Figure S3. Principal component analysis of network-level topological features in the male (A) and female (B) gut microbial co-occurrence networks.
Figure S4. Network-level topological features in the microbial subnetwork of the male and female gut with the development of colorectal carcinoma.
Table S1. Network topological properties used in this study.
Table S2. Topological features of male and female meta-community co-occurrence networks.
Supplementary Methods.