A study of the effects of diet on human gut microbial community structure and mercury metabolism

A thesis submitted to the

Faculty of Graduate and Postdoctoral studies

In the partial fulfillment of the Requirements

For the Degree of Masters

In the Environmental sustainability Graduate Program

Institute of Environment/Faculty of Biology

University of Ottawa

Ottawa, Canada

By

RIA SAHA

Supervisors: Dr. Alexandre Poulain & Prof. Laurie Chan

Table of Contents:

Abstract:

Background: Recent research showing how dietary interventions substantially influence the potential presence of widespread and stable bacterial core phyla in the human colon has garnered a considerable amount of attention. Because the human gut can play a major role in host health, there is currently some interest in observing how diet influences human gut microbial composition and how changes in diet affect the potential for gut microbiota to transform mercury.

This study aims to discover how different kinds of diet affect the nature and magnitude of microbial Hg transformations in the human gut environment.

Methods: Fecal samples have been collected from 5 human male individuals at University of Ottawa and stored at -80ºC for further investigation. Using high throughput DNA amplicon sequencing targeting the 16s rRNA V4 region, we investigated the microbial community structure of the gut in 5 healthy male. Mercury biotransformations in the pooled fecal sample have been carried out using stable isotopes of mercury ($^{198}HgCl_2$ and $Me^{199}HgCl$) and analysis was conducted by using inductively coupled plasma mass spectrometry (ICP-MS).

Results and conclusions: We were not able to detect any significant Hg methylation or MeHg demethylation. We suspect this is due to Enterobacteria dominating the microbial community structure after 96h; Although Enterobacteria are part of the typical microbiota of a healthy individual, they do not possess genes required for Hg methylation. As such, our microbial data support our chemical analyses. We were not able to identify whether a change in diet affected Hg transformations in the human gut environment.

Keywords: Mercury (Hg), gut microbiota, transformation

Résumé:

Contexte: Des recherches récentes montrant comment les interventions alimentaires influent de manière substantielle sur la présence potentielle de phylum de base bactérienne répandue et stable dans le côlon humain ont attiré beaucoup d'attention. Parce que l'intestin humain peut jouer un rôle majeur dans la santé de l'homme, il existe actuellement un certain intérêt à observer comment le régime influence la composition microbienne de l'intestin humain et la façon dont les changements dans le régime affectent le potentiel du microbiote intestinal de transformer le mercure.

Cette étude vise à découvrir comment les différents types de régime affectent la nature et l'ampleur des transformations microbiennes de Hg dans l'environnement intestinal humain.

Méthodes: Des échantillons de matières fécales ont été recueillis auprès de 5 individus humains humains à l'Université d'Ottawa et stockés à -80 ° C pour une enquête plus. En utilisant un séquençage d'amplicon d'ADN à haut débit ciblant la région V4 de l'ARNr de 16s, nous avons étudié la structure de la communauté microbienne de l'intestin chez 5 individus masculins en bonne santé à l'Université d'Ottawa. Les biotransformations de mercure dans l'intestin humain ont été réalisées à l'aide d'isotopes stables de mercure (198HgCl2 et Me199HgCl) et l'analyse a été réalisée en utilisant ICP-MS.

Résultats et conclusions : Cependant, nous n'avons pas été en mesure de détecter une méthylation d'Hg significative ou une déméthylation de MeHg. Nous soupçonnons que cela est dû aux entérobactéries dominant la structure de la communauté microbienne après 96h; bien que les entérobactéries appartiennent au microbiota typique d'un individu sain, elles ne possèdent pas des gènes requis pour la méthylation de l'Hg. En tant que telles, nos données microbiennes supportent nos analyses chimiques. Nous n'avons pas été en mesure d'identifier si une modification du régime a affecté les transformations d'Hg dans l'environnement intestinal humain.

Mots clés : Mercure (Hg), microbiote intestinal, transformation

List of Figures

List of Tables

List of Abbreviations:

ANOVA	Analysis of Variance
$CuS0_4$	Copper sulfate
CH_2Cl_2	Dichloromethane
Co	Cobalamin
DGGE	Denaturing gradient gel electrophoresis
FAFV	Firmicutes, Actinobacteria, Fusibacteria, Verrucomicrobia
Gastrointestinal tract	GI tract
GC-AFS	Gas chromatography-Atomic fluorescence spectroscopy
HCl	Hydrogen chloride
Hg^{II}/Hg^{2+}	Mercuric mercury
$HgCl_2$	Mercuric chloride
HgO	Mercuric oxide
HgT	Total mercury
ICP-MS	Inductively coupled plasma - mass spectrometry
KBr	Potassium bromide
KCN	Potassium cyanide
KOH	Potassium hydroxide
MANOVA	Multivariate analysis of variance
MeHg/CH_3Hg	Methylmercury
MeHgCl	Methyl mercuric chloride

$Na_2S_2O_3$	Sodium thiosulfate
$NaBEt_4$	Sodium tetraethylborate
ND	Non-digestible
NPSHS	Non-protein sulfhydryl compounds
PBS	Phosphate buffered saline
PCA/PCoA	Principal coordinate analysis
PC	Principal coordinate
PCR	Polymerase chain reaction
QIIME	Quantitative insights into microbial ecology
RS	Resistant starch
SCFA	Short chain fatty acid
SH	Sulfhydryl
SHIME	Simulated Human Intestinal Microbial Ecosystem
Vit	Vitamin

Acknowledgements:

I would like to gratefully acknowledge various people who have been journeying with me in recent years as I have worked on this thesis.

I would like to thank my supervisors, Dr. Alexandre Poulain and Prof. Laurie Chan, for taking me on as a student. They were extremely helpful throughout the year of my research. Their advice on scientific research and writing has helped me advance both my knowledge and my confidence in the field.

The students and staff at the Institute of Environment/Faculty of Biology have provided me with ongoing support, and technical assistance throughout my degree program. Thank you for always scheduling around my lab work.

I would like to thank Mr. Galen Guo, Mr. Matti Ruuskanen, Dr. Dipak Rana and Dr. Emmanuel Yumvihoze - four incredibly intelligent persons who guided me through the process of my project. They taught me how to conduct experiments, provided me with resources, and answered countless questions.

Finally, my thesis would not have been completed without the long distance support of my family. Without their love and support over the years, none of this would have been possible. They have always been there for me and I am thankful for everything they have helped me to achieve.

Thank you!

Introduction:

Increasing evidence shows that varied nutrient loads and nutritional ingredients influence the microbial community structure and production of metabolites by gut microbiota, which has also been shown to be involved in mercury toxicity (Landry et al., 1979). The exact mechanism(s) are, thus far, unclear. Microbes modulate the toxicity of mercury (Hg) through methylation of mercuric mercury (Hg^{II}) and demethylation (i.e. detoxification) of methylmercury (MeHg). (Barkay et al., 2003; Glimour et al., 2013; Parks et al., 2013; Smith et al., 2015). MeHg is completely absorbed from the mammalian gut rapidly and this is one of the unusual characteristics of toxic heavy metals (Miettinen et al., 1971; Walsh et al., 1988). The distal gut harbors greater than 10^{11}-10^{12} organisms per ml of luminal content, making it one of the most densely populated ecosystems and an important reservoir for Hg cycling and MeHg metabolism (Rotherburg et al., 2015). Several *in vivo* and *in vitro* studies have confirmed gut microbiota and different diet modulate enterohepatic cycling of MeHg and its absorption into tissues. Animals treated with antibiotics have shown a reduced decomposition of MeHg in the large intestine compared to controls (Seko et al., 1981), and an increase in the half-time of MeHg elimination (Rowland et al., 1984), thus implicating gut microbes in MeHg metabolism. Several public health studies highlighted the role of diet in influencing Hg absorption and/or toxicity. Studies by Passos et al. (2007) and Rowland et al. (1983, 1986) identified that co-consumption of high fibre foods, including wheat and fruit, were associated with lower absorption of MeHg into tissues. Microbial MeHg detoxification involves two primary microbial genes which are, *MerA*, the mercuric reductase, and *MerB*, the organomercurial lyase (Barkay et al., 2003). Genes encoding these proteins have been successfully recovered from human and non-human primate feces, further verifying potential decomposition of Hg species by gut microbiota (Liebert et al., 1997). Gut microbes are potential microrganisms responsible for methylation of Hg. Until now, one commensal methanogen (*Methanomassiliicoccus luminyensis*) has been isolated from human feces (Dridi et al., 2012) containing the gene cluster (HgcA and HgcB) responsible for Hg methylation (Parks et al., 2013). However, intestinal bacterial methylation was observed by some researchers (Rowland et al., 1975), but not others (Zhao, 2013). Despite the recognition that diet and nutrition can influence a population's susceptibility to the effects of MeHg (NRC, 2000), dietary information has not been systematically collected and studied in most epidemiological studies examining the effects of MeHg exposure (Chapman and Chan, 2000). Although a number

of controlled experiments estimated the effects of specific nutrients on Hg absorption and/or toxicity (Calabrese, 1978; Levander and Cheng, 1980; Imura and Naganuma, 1985; Whanger, 1992; Peraza et al., 1998; Lapina et al., 2000; Rao et al., 2001; Rao and Sharma, 2001; Usuki et al., 2001; Afonne et al., 2002), *in vitro* studies determining the role of diet affecting Hg metabolism in free living populations are rare.

Due to these microbiota-host and diet interactions, strategies using dietary interventions can be used to tackle, prevent or even treat worldwide epidemics related to mercury toxicity. To this end, more information is required on how dietary compounds differently and rapidly affect the composition and activity of the gut microbiota affecting Hg metabolism.

Studies performed in humans are the ideal way to assess these effects. However, studies involving human subjects are limited due to ethical concerns, difficulties in sampling and are usually very expensive (Aguirre et al., 2015). An alternative is to perform *in vitro* studies, which in addition, offer certain advantages like reproducibility assessment studies where the potential lethality restricts *in vivo* testing including toxicity of metals (e.g. mercury, arsenic, cadmium, chromium, lead, etc.), and biotransformation of drugs or toxic compounds (e.g., polycyclic aromatic hydrocarbons (PAHs), polynuclear aromatic hydrocarbons (PNAs), etc.) on the gut microbiota.

This pilot study involves the collection of freshly voided fecal samples from five healthy human individuals because human feces are considered as the largest reservoir of human gut microbiota. The batch fermentation system is developed by the Chan and Poulain Laboratories, and represents a powerful, reproducible and validated tool for monitoring fermentation of dietary compounds using human feces as inoculums. It is accompanied by assessment of modulation of mercury toxicity by gut microbiota. The overarching hypothesis of this project is that ***diet affects the nature and magnitude of microbial Hg transformations in the human gut environment***. Using *in vitro* studies, we made the following predictions:

1. **The microbial community of human gut microbiota in batch culture will undergo changes upon dietary interventions within 4 days**
2. **A high carbohydrate diet will increase the relative abundance of targeted microbial communities like Bifidobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Methanoarchea (carbohydrate fermenters)?**
3. **Cultures provided with high protein on low fat/plant polysaccharides will exhibit high proportions of *Bacteroides* spp.**
4. **Diets supplemented with a high amount of fat will exhibit high amounts of Proteobacteria, and Fusobacteria**
5. **Diets low in polysaccharide (mainly enriched with sulfated mucins, and glycans) will increase the *Bacteroides* spp. encoding sulfatases that are accompanied by significantly higher proportional levels of *Desulfovibrio* spp., this species is commonly known sulfate reducing bacteria (SRB) that colonizes the human gut**
6. **Methylmercury transformations will be modulated by changes in diet due to changes to the microbial community of the gut**

LITERATURE REVIEW:

Microbial ecology of human gastro-intestinal tract:

Gut microbiota are a part of the human gastrointestinal tract, a complex ecological community that carries a diverse and abundant group of microorganisms. Human gut microbiota is a key component in maintaining gastrointestinal tract (GI tract) homeostasis (Tap et al., 2009). Microbes can have a widespread impact on diverse aspects of a host's physiology from the effects on the host's digestive tract and its metabolism and host's immune status and function (Sommer and Bäckhed, 2013). Microbiota harbor complex consortia of micro-organisms, which colonize the length of the gut (Steer et al., 2000). The human gut functions as a chemostat, a continuous culture system for microorganisms (mostly bacteria) in which fresh nutrients enter the system and cultured microorganism leave at a constant rate (Sonnenburg et al., 2004). The human intestinal microbiota is composed of 10^{13} to 10^{14} microorganisms whose collective genome ("microbiome") outnumbers the genes of the human genome by a factor of 100 times (Gill et al., 2006). Host digestive physiology, including the pH of the gut and the presence of bile

acids, and components of the innate immune response, such as defensins and immunoglobulin A, are what control the colonization of microbiota. This is done by imposing a selective pressure – from early life that is which strains can colonize the gut (Hooper et al., 2012). At birth, the GI tract essentially remains germ free, with initial colonisation occurring during birth or shortly afterwards (Steer et al., 2000). The composition of the microbiota undergoes substantial changes at three stages in life: from birth to weaning; from weaning to attaining a normal diet; and at old age, a relatively stable climax community is established (Flint et al., 2012). Facultative anaerobes initially colonize the gut during birth (Eggesbø et al., 2011). Colonization of these anaerobes creates an anaerobic condition that promotes the growth of obligate anaerobes (initially *Bifidobacterium* and *Bacteroides* spp.) within about two weeks (Flint et al., 2012). Naturally born infants become inoculated (Koenig et al. 2011; Marathe et al. 2012) by the mother's vaginal or fecal microbiota during delivery (Karlsson et al., 2011), and those born by caesarian section are initially colonized by bacteria from the environment and skin (Dominguez-Bello et al., 2010). Sequence based characterization of 16*S* ribosomal RNA gene of the distal gut and fecal microbiota published to date indicates that gut microbiota is highly selective in nature (Gill et al., 2006). Around 90% of all taxa belong to two predominant phyla: Bacteroidetes and Firmicutes. Other phyla in the human distal gut constitute Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia (Fig. 1) (Guarner, 2015). Improvements in different 16S rRNA gene based enumerations and technologies like proteomics and metabolomics has identified significant differences in diversity and function between healthy adults (Eckburg et al., 2005; Ley et al., 2005). These differences may contribute to variations in normal physiology between individuals or may predispose them to particular diseases (Gill et al., 2006). Microbial abundance gradually increases from the stomach ($<10^3$ bacteria/g) towards the colon (10^{11} bacteria/g). The microbial communities that colonize different regions of the human gut influence several aspects of health over a lifetime (Flint et al., 2012). In a healthy individual, a vast majority of these microbes (10 to 100 trillion) reside in the GI tract, with the greatest number inhabiting the distal gut (Gill et al., 2006). These microbes potentially contribute nutrients and energy to the host via the fermentation of non-digestible dietary components in the large intestine. This establishes a balance between the host's metabolism and immune system (Flint et al., 2012) (Fig. 2). In the small intestine, bacterial abundance and diversity are limited due to fast transit time and digestive secretions such as bile acids (Steer et al., 2000). The

movement of gut content slows down in the lower part of the gut where sizeable microbial populations are observed (about 106 CFU/ml) (Steer et al., 2000). Microbial populations can attain a maximum level of 10^{12} CFU/g lumen contents (Conway, 1995; Gibson et al. 2000).

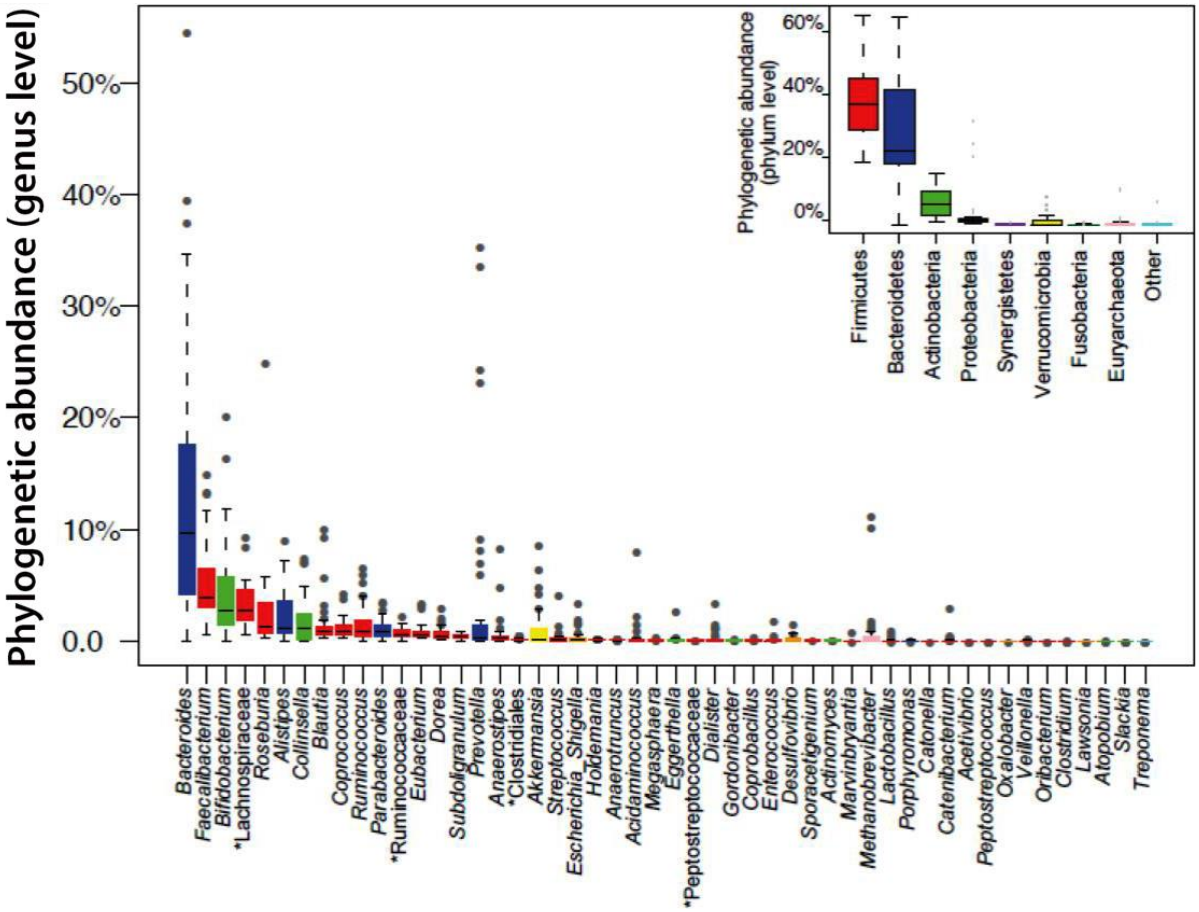

Figure 1: A genus abundance variation box plot for the 30 most abundant genera of the human gut microbiota as determined by metagenomic sequencing of human fecal samples. Genera are colored by their respective phylum (see inset for color key). The inset shows the phylum abundance box plot. Genus and phylum level abundances were measured using reference-genome-based-mapping. Source: Arumugum et al., 2011.

Although human microbiota varies significantly between individuals, family members share more similar microbiota than non-related individuals (Marathe et al., 2012). The human GI

tract microbiota appeared to be dominated predominantly by very few selected phyla when compared with other complex ecosystems such as soils and oceans (Cole et al., 2005). Nonetheless, these are highly diverse and complex at the level of 'phylotype' (Tap et al., 2009). The stability of the gut microbiota establishes 'colonization resistance' by limiting the capacity of invading micro-organisms, including pathogens, to colonize the gut (Hentges, 1992). In a healthy individual, the intestinal immune system exists in a state of homoeostasis with the microbiota, thus reflecting the beneficial role of the gut microbiota and the evolution of 'indigenous' micro-organisms alongside their human host (Conway, 1995).

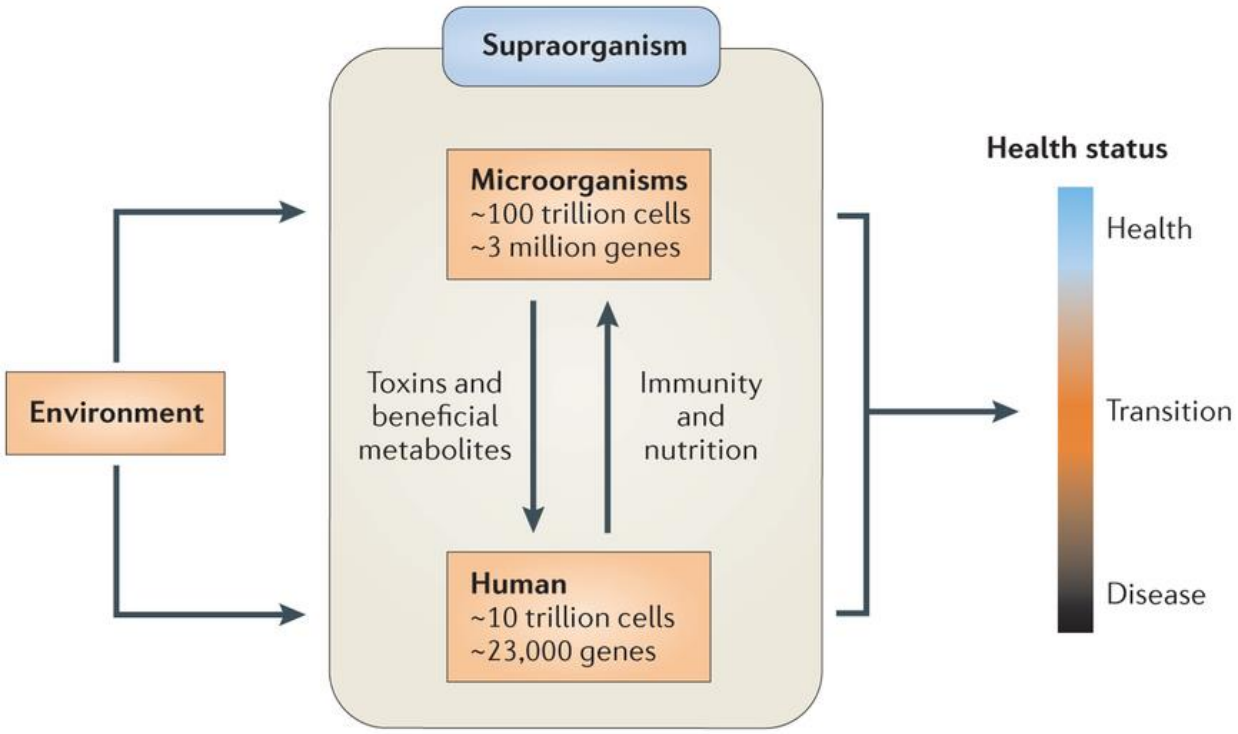

Figure 2: Human health is influenced by interactions among the gut microbiota, the host and the environment. Source: Zhao, 2013.

Gut environment:

The gut environment varies markedly across different anatomical regions of the human host in terms of physiology, digest a flow rates, substrate availability, host secretions, pH and oxygen

tension (Flint et al., 2012). The large intestine, which is characterized by slow flow rates and neutral to mildly acidic pH, harbours by far the largest microbial community (dominated by obligate anaerobes). Significant differences in gut environment occur between the proximal and distal regions and, even more locally, between the gut lumen and surfaces (Flint et al., 2012). The small intestine provides a challenging environment for microbial colonizers due to its fairly short transit times (3-5 h) and high bile concentrations (Boojink et al., 2010; Zoetendal et al., 2012). Colon pH varies from mildly acidic conditions in the proximal colon to more neutral pH distally (Flint et al., 2012). pH values <6.0 at short chain fatty acids concentrations typical of the colon limits the growth of *Bacteroides* spp. (50-100 mM) (Duncan et al., 2002). Firmicutes are more tolerant of acidic pH, which provides them with a competitive advantage to colonize at low pH, which results from active substrate fermentation (Flint et al., 2012). A substantial shift in species composition and metabolic outputs of human intestinal microbiota has been observed between a pH of 5.5-6.5 in an *in vitro* system (Walker et al., 2011).

Oxygen concentrations influence the spatial distribution of the microbiota in the human gut (Swidsinki et al., 2008). Colonization of facultative anaerobes makes the colonic lumen highly anaerobic (Eh of ~250 mV). This results because these anaerobes by consuming the available oxygen (Flint et al., 2012). Most of the colonic bacteria are strictly anaerobes (like Bacteroidetes, Proteobacteria, Firmicutes, etc.) that fail to grow in $>5 \times 10^{-3}$ atm oxygen (Flint et al., 2012). Although most of the colonic microbiota are strict anaerobes, the growth of some facultative anaerobes like *Bacillus fragilis*, and *Faecalibacterium prausnitzii* get stimulated by very low concentrations of oxygen (Flint et al., 2007; Khan et al., 2012).

Human gut flora and diet:

Diet plays a crucial role in shaping the human gut microbiota. Different phenotypic analysis (metabolomics) and compositional (metagenomics) assessments indicated that diet is a fundamental driver of gut microbial diversity and stability. This arises between 2 and 4 years of age (Lozupone and Knight, 2005). In recent times, interest in ‘optimizing’ the intestinal microbiota composition by dietary modulation is gaining a considerable amount of attention

(Graf et al., 2015). Recent research showing how dietary interventions substantially influence the potential presence of widespread and stable bacterial core phylogroups in the human colon has made substantial progress in understanding and identifying the pivotal role of diet on shaping the human gut. Dietary effects on gut microbiota and health are potentially confounded by a variation in host genotypes and various environmental factors and exposures (Xu and Knight, 2014). Nevertheless, recent studies suggest that of all the exogenous factors affecting the gut microbiome, a long term diet appears to have the largest impact to date (Xu and Knight, 2014). For example, two co-evolution studies of mammals and their gut microbiota has found that both gut microbiota composition and functions are adapted to their type of diet (herbivorous, carnivorous and omnivorous) (Ley et al., 2008; Muegge et al., 2011). Microbial activities in the large intestine are considered to play an important role in the maintenance of gut health and in the etiology of gut disease in humans (Flint et al., 2007). Comparative analyses suggest that diet is a major environmental factor contributing to gut microbial variation across mammalian species (Muegge et al., 2011). Diet also shapes the gut microbiota within a species as evidenced by longitudinal studies of the black howler monkey gut microbiota (Amato et al., 2013, 2015) and dietary perturbation experiments in wild-caught mice and fish (Bolnick et al., 2014; Wang et al., 2011). Zhao (2013) reported that 57% of variation in the composition of gut microbiota was driven by dietary change, while only 12% was due to genetic differences (Zhang et al., 2010). The impact of dietary changes upon microbial metabolism occurs through several inter-related mechanisms (Fig. 3) (Louis et al., 2007). Metabolism is tightly regulated within each individual species of gut bacterium (Flint et al., 2012). At a macroscopic level, however, the microbiota support a common set of metabolic pathways assembled in a trophic chain common to all healthy individuals (Macfarlane and Gibson, 1994). This typically follows a common metabolic pathway across all individuals, involving fermentation of dietary compounds and endogenous substrates, followed by host absorption and excretion of short chain fatty acids (SCFAs) (like acetate, propionate, and butyrate) and gas (Tap et al., 2009). Alterations in dietary composition result in both quantitative and qualitative changes in the supply of substrates to the large intestinal microbiota (Louis et al., 2007). Alternative substrates can lead to the production of different metabolic products as a result of fermentation via different metabolic pathways, while the same substrates can be processed via different routes depending on their rate of supply, or the physiological environment of the bacterial cell (Macfarlane and Macfarlane 2011; Scott et al.,

2006). Dietary components that do not undergo digestion by endogenous enzymes in the upper gastrointestinal tract become available as substrates in the large intestine. Some of these dietary components are 'non-digestible' (ND) dietary carbohydrate substrates which include resistant starch, plant cell wall material and oligosaccharides (Cummings and Macfarlane, 1991). Many secondary plant metabolites ingested with the diet, such as polyphenolic substances, may also reach the large intestine and undergo bacterial transformations (Flint et al., 2012). The microbial processes occurring in the gastrointestinal tract are heavily influenced by dietary ingredients, such as, prebiotics (Grootaert et al., 2011) and probiotics, (Goldin, 2011), or by drugs, such as antibiotics (Jernberg et al., 2010). Evidence from *in vivo* studies (monitored by fecal sampling) with prebiotics (mainly oligofructose) suggest that sustained changes in the supply of ND dietary carbohydrate can lead to shifts in the species composition of the colonic bacterial community (Gibson et al., 2000). The need to control for dietary variation when evaluating microbial shifts in composition is a priority in future research. However, there are several challenges and limitations regarding this effort as human studies normally provide fecal samples which reflect the microbiota of the distal colon, but do not allow access to the microbiota of the actual site of food fermentation (caecum and proximal colon) (Graf et al., 2015). Further, human studies in this field usually have low numbers of participants primarily due to ethical concerns and participants differ significantly in their dietary behavior and life-styles (Graf et al., 2015). Pig model systems that have similar gastrointestinal tracts and diets to humans and humanized germ free mice provide comparative approaches for examining microbiota interactions with specific diets (Tremaroli and Bäckhed, 2012). Though results from animal experiments cannot be extrapolated directly to humans, they provide proof of concept and pave the way for further research focusing on human dietary interventions. However, deeper mechanistic understanding of diet's role in host microbial function imminently depends on integrating *in vitro* and *in vivo* models into a systems level framework for understanding the functional interactions between diet composition, gut microbiota and host metabolism (Borenstein, 2012; Dimitrov, 2011).

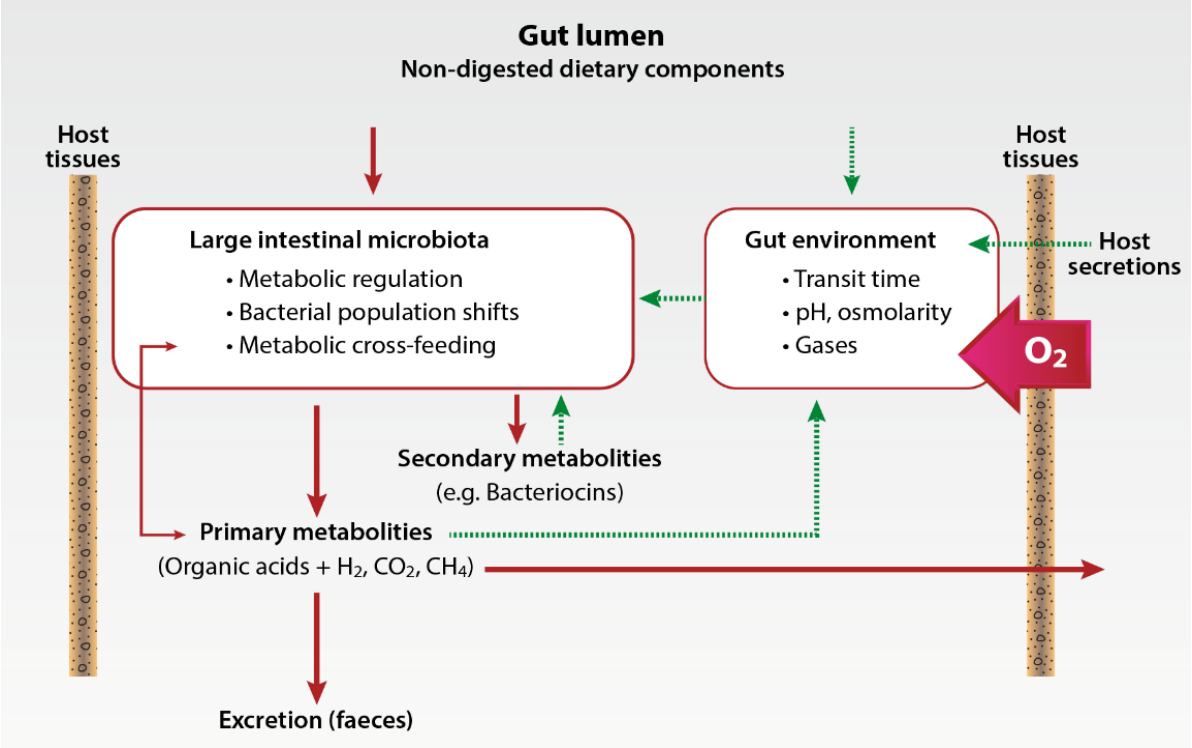

Figure 3: Schematic diagram of the gut microbial ecosystem. Metabolic flows are shown with solid arrows and other influences are shown with dashed arrows. Source: Louis et al., 2007.

In summary, the effects of dietary differences on gut microbial communities are discussed below-

Specific foods-

Whole grain products:

Whole grain products are characterized by the presence of a high amount of dietary fibre (Graf et al., 2015). Carvalho-Wells et al. (2010) reported increased levels of *Bifidobacteria*, and *Lactobacillus/Enterococcus* group in the feces of volunteers who consumed maize-based whole grain products, whole grain rich cereals and placebo cereals, respectively.

Vegetables and Legumes:

A growing body of scientific evidence indicates that dietary modulation, by ingestion of prebiotics, such as soygerm powder which contains isoflavones (dietary anti-carcinogen), might shift the microbial ecosystem toward higher concentrations of lactic acid–producing bacteria, which may be beneficial for the host (Fooks et al., 1999; Fuller and Gibson 1997; Roberfroid, 1998). A study by Gibson et al. (2000) found that dietary modulation promotes colonic health by lowering the gut pH of metabolic products.

Fruits and nuts:

Vendrame et al. (2011) reported an increased amount of *Bifidobacterium* spp. and *Lactobacillus acidophilus* in feces of volunteers after a daily consumption of blueberry drink and placebo treatment, respectively. Another study observed an increase in abundance of Bifidobacterium genus after consumption of red berries (Queipo-Ortuño et al., 2012). The Mediterranean diet, including relatively large intakes of vegetables, fruits, olive oil and nuts may increase the proportions of *Bifidobacterium*, *Enterococcus*, *Bacteroides* and *Prevotella* and may decrease the abundance of pathogens like *Clostridium perfringens* (Lopez-Legarrea et al., 2014). Tan and O'Toole (2015) found that 30% of the gut microbial species underwent significant changes after the administration of peanut based therapeutic food composed of health promoting beneficial bacteria like *Bifidobacterium, Lactobacillus,* and *Faecalibacterium prausnitzii*.

Dietary pattern-

Western diet:

In Western countries people usually rely on diet which contains low fiber, high fat and refined carbohydrates compared to a traditional rural diet which is generally high in fiber (Graf et al., 2015) (Fig. 4). A study by Watanabe & Koessel (1993) indicates that consumers of a western type diet have higher proportions of *Clostridium perfringens* and other bacteria strains with 7-a-dehydroxylase activity. African children have increased amounts of *Prevotella* sp. compared to European children and African Americans (De Filippo et al., 2010). Researchers (De Filippo et

al. 2010; Ou et al., 2013; Schnorr et al., 2014) reported that several African populations have microbiota enriched with *Succinivibrio* and *Treponema.* European children who are fed a typical western diet (De Flippo et al., 2010) are relying on a such a diet which harbors a significant over-abundance of Firmicutes like *Acetitomaculum* and *Feacalibacterium,* as well as Enterobacteriaceae (*Shigella* and *Escherchia*) (De Filippo et al., 2010).

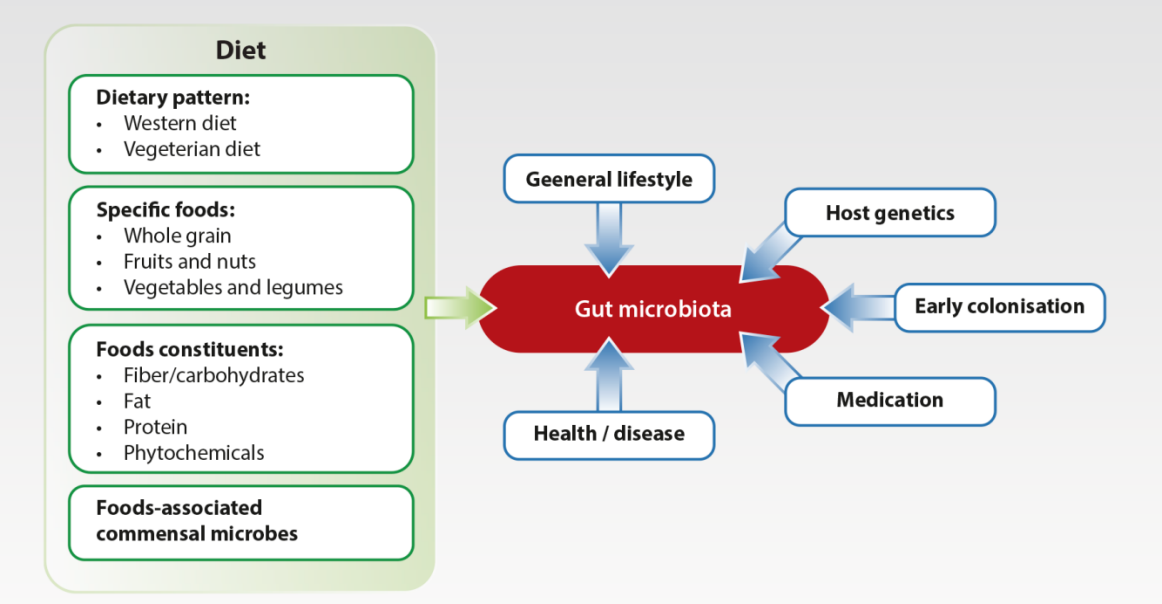

Figure 4: Factors which influence the composition of the gut microbiota, with special focus on diet. Source: Graf et al., 2015.

Vegetarian diet:

Vegetarian diet decreases the concentration and diversity of *Clostridium* cluster IV, which are beneficial butyrate-producing bacteria (Listz et al., 2009). Matijašić et al. (2014) found increased proportions of *Bacteroides/Prevotella* group, *Bacteroides thetaiotaomicron*, *Clostridium clostridioforme*, and *Faecalibacterium prausnitzii* among vegetarians. Polymerase chain reaction (PCR) with denaturing gradient gel electrophoresis (DGGE) finger printing analysis conducted by Listz et al. (2009) revealed that vegetarians carry higher levels of *Bacteroides* and a lower

abundance of *Clostridium* cluster IV, especially *Faecalibacterium* sp. and *Ruminococcus* sp. as compared to omnivores.

Food constituents-

Carbohydrates/Plant polysaccharides:

Both *Bifidobacterium longum* (Schell et al., 2002) and *Bacteroides thetaiotaomicron* (Xu et al., 2003) dedicate at least 8% of their genomes to carbohydrate transport and metabolism functions.

Dietary fiber:

Plant derived foods carry high amounts of dietary fiber, such as resistant starch (RS), pectin, oligosaccharides and inulin, most of which are utilized by intestinal bacteria (Eswaran et al., 2013). Complex carbohydrates like dietary fiber are metabolized by the colonic microbiota to simpler forms of oligosaccharides and monosaccharides. Oligosaccharides are then further fermented to SCFA, which function both as an energy source and as a signalling molecule (Bazzocco et al., 2008). Insoluble plant cell wall material consists of high amounts of cellulose, arabinogalactan, xyloglucan, β-glucan, mannan, pectins and lignin (Louis et al., 2007). These complex polymers are intimately associated in the plant cell wall, and are degraded by a cascade of microbial hydrolases, esterases and lyases present in the human colon (Louis et al., 2007). Diversity of the microbiota and their metabolic interactions are directly correlated with the content and type of dietary fiber intake (Tremaroli et al., 2012). For instance, De Flippo et al. (2010) found an increase in abundance of phylum Bacteroidetes, mainly *Prevotella* and *Xylanibacter* in rural African children consuming diets with high amounts of fiber, starch, plant polysaccharides with very low fat and animal protein. Rumen gut anaerobes like *Ruminococcus flavefaciens* are responsible for plant cell wall degradation (Flint et al., 2007), but until now few have been isolated from human fecal samples (Robert and Bernalier-Donadille, 2003). Hemicelluloses and pectins which are present in the cell wall matrices are partially released in the soluble form by the cell wall degrading gut bacteria (Flint et al., 2007). Flint et al., (2007) showed that certain species of bacteria namely, *Bacteroides ovatus*, *Prevotella bryantii*, and *Roseburia intestinalis* in the human colon can utilize complex substrates like xylans, heterogeneous polymers of β-1, 4-linked xylose residues substituted with acetyl, arabinosyl and

glucuronic acid residues. Lactulose, a synthetic disaccharide, has been observed to stimulate the bifidogenic activity in the human gut (Modler, 1994; Tuohy 2000; Tuohy et al., 2001; Ziemer and Gibson, 1998). Populations in the Hadza community who are solely dependent on high fiber foods for their diet have been found to have extensive amounts of *Prevotella*, *Treponema* and unclassified Bacteroidetes, as well as a specific set of Clostridiales (Schnorr et al., 2014).

Pectin:

Pectin is composed of linear long chains of alpha- 1, 4 –linked galacturonic acid residues which are known as smooth pectin or could be branched forming rhamnogalacturonans known as hairy pectin (Flint et al., 2007). Since the structure of pectin varies across different sources of food, a simple comparison of the impact of pectin-rich diets on microbiota composition usually yields different results (Sajilata et al., 2006). For example, a 3 week study conducted by Dongowski et al. (2002) noticed an increase in *Bacteroides* spp. in the colon of rats fed a diet containing 6.5% citrus pectin, while a 4 week study with rats given a 7% apple pectin rich diet showed a higher abundance of *Anaeroplasma*, *Anaerostipes*, and *Roseburia*, with a decrease in the number of *Alistipes* and *Bacteroides* spp (Licht et al., 2010). Pectin from apple intake was observed to increase the proportions of *Bifidobacterium, Lactobacilli* and *Streptococcus* while reducing Enterobacteriaceae, *Pseudomonas* and some lecithinase-positive *Clostridia sp.* (Shinohara et al., 2010).

Resistant starch:

Starch is made out of two distinctive glucose polymers, amylose and amylopectin, and a part of these can pass undigested into the internal organ making a dietary fiber called resistant starch (RS) (Hamaker and Tuncil, 2014). RS has four subgroups: (1) RS1, physically inaccessible starch present in whole grains or partially milled grains and legumes; (2) RS2, tightly packed and relatively dehydrated granular starch; (3) RS3 is a cooked and re-associated, or retrograded form of starch (principally amylose) where host enzymes have limited access; and (4) RS4 is a chemically or enzymatically modified starch (Sajilata et al., 2006). Due to their structural and accessibility differences, different RS types within type favor different resident bacteria in the human gut (Sajilata et al., 2006). The fraction of RS starch which does not get digested by the host amylases, and reaches the colon to get metabolized by the colonic bacteria (Flint et al.,

2007). RS2 consumption is associated with an increase of *Ruminococcus bromii* and *Eubacterium rectale* (Martínez et al., 2010). Studies by Tan and O'Toole (2015) found that the amount of RS4 consumption is directly proportional with Actinobacteria and Bacteroidetes; it has found to have a significant impact on *Bifidobacterium*, resulting in a ten-fold to 18-30% in gut microbiota. The starch degrading system of the gram-negative bacterium *B. thetaiotaomicro* encodes multiple gene products that attach, hydrolyse and translocate starch molecules (Xu et al., 2003). RS molecules increase the abundance of certain high G+C gram-positive *Bifidobacterium spp*. which are amylolytic and adhere to starch granules, on a specific cell surface protein (Crittenden et al., 2001).

Cellulose:

Cellulose possesses a unique molecular structure in which the molecules are linked through intermolecular and intramolecular hydrogen bonds and form crystalline linear aggregates (microfibrils) (Hamaker and Tuncil, 2014). Due to its unusually complex structure, it is not usually completely fermented in the human gut (Zhang and Lynd, 2004). Cellulose consumption increases the abundance of *Clostridium sp*, *Eubacterium sp*, and *Ruminococcus sp*., (Robert and Bernalier-Donadille, 2003; Montgomery, 1988; Wedekind et al., 1988) and *Bacteroides sp*. (Betian et al., 1977). A human study documented that the structure of the cellulose degrading mechanism differs across individuals, largely depending on the methane status of the subjects (Chassard et al., 2010). Non-methane excreting subjects mainly contain Bacteroidetes as dominant cellulose degraders, while Firmicutes usually dominates in methane-excreting individuals (Chassard et al., 2010).

Hemicelluloses:

Hemicelluloses are a major group of dietary fibers which includes arabinoxylans, xyloglucans, glucomannans, galactomannans, and β-glucans (Hamaker and Tuncil, 2014). Arabinoxylooligosaccharides are a kind of prebiotics, which have been shown to stimulate the growth of *Bifidobacteria* in the human colon (Hamaker and Tuncil, 2014). Studies utilizing a compartmentalized simulator of the human intestinal microbial ecosystem have shown that arabinoxylooligosaccharides degradation in the colon is compartment specific (mainly occurring in the transverse colon) (Grootaert et al., 2009) and highly dependent on its average degree of

polymerization (Hamaker and Tuncil, 2014). Arabinoxylooligosaccharides with an average degree of polymerization of 29 have been shown to increase the abundance of *Bifidobacteria* in the ascending colon, lactobacilli in both ascending and transverse colons, and groups of *Clostridium coccoides–E. rectale* in the descending colon (Sanchez et al., 2009). Studies with humanized mice indicate that Arabinoxylan hydrolyzates with a degree of polymerization of 60 or higher, decreased the cecal abundance of *Clostridium* clusters I/XI/XV and Verrucomicrobia, while significantly increasing cecal Actinobacteria (Van den Abbeele et al., 2011). The molecular size of β-glucan is a significant predictor of colonization of bacterial groups in the human gut. For example, an *in vitro* study inoculated with β-glucan hydrolyzates of molecular masses 137, 150, and 172 kDa showed an increased abundance of the *Bacteriodes– Prevotella* group at 24 h, but no significant increases were observed when provided with larger-size hydrolyzates (230 and 243 kDa) (Hughes et al., 2008).

Inulin:

Inulin consumption results in increased abundance of butyrate producing bacteria like *E. rectale, Roseburia intestinalis,* and *Anaerostipes cacca* (Barcenilla et al., 2000; Schwiertz et al., 2002; Duncan et al., 2002), while decreasing the abundance of *Akkermansia muciniphila* in the cecum of humanized rats (Van den Abbeele et al., 2011). Costabile et al. (2010) reported a significant increase of *Bifidobacterium,* lactobacilli/enterococci among healthy volunteers upon inulin consumption. Furthermore, this was also observed to increase the abundance of the *Atopobium* group with a significant reduction of the *Bacteroides/Prevotella* group (Costabile et al., 2010).

Fat:

Human mediation information advances the idea that dietary fat, by implication, balances intestinal microbiota arrangements. The dietary fat does this by means of its effect on bile acid secretion and also on bile acid composition (Graf et al., 2015). Wu et al. (2011) reported that the *Bacteroides* enterotype is positively correlated with the intake of saturated fats, while the *Prevotella* enterotype is inversely associated with the total intake of dietary fat. A high fat diet in a mouse model was observed to trigger the genes involved in signal transduction, cell motility,

membrane transport, replication and repair within the phyla of Proteobacteria, Bacteroidetes and Firmicutes (Hildebrandt et al., 2009).

Proteins:

Russell et al. (2011) reported an increase in branched-chain fatty acids, a decrease in butyrate, and a decrease in *Roseburia/Eubacterium* numbers after a 4-week period of protein diet given to obese men. Doré and Blottière (2015) documented that a diet rich in animal proteins and fat, which is typical of food intake in western societies, will favor the *Bacteroides* enterotype.

Foods associated commensal microbes- Prebiotics:

A prebiotic is a nonviable food segment that presents a medical advantage to the host by altering the microbiota (AGNS, 2007). It acts as a modulator of the diversity of autochthonous microbiota (Doré and Blottière, 2015). ND food ingredients like plant cell wall polysaccharides (cellulose, xylan and pectin) have prebiotic properties that stimulate specific microbes to improve metabolic regulation in the host body and are now increasingly being introduced into the western diet (Murphy et al., 2013). Prebiotics, such as galactosaccharides, together with inulin and their fructo-oligosaccharides derivatives, have shown to modify species composition of the colonic microbiota (Macfarlane and Macfarlane, 2011). Buddington et al. (1996) reported that consumption of fructo-oligosaccharide (FOS) (4 g/d), a prebiotic, produced an increase in *Bifidobacteria* and *Faecalibacterium prausnitzii* (Ramirez-Farias et al., 2009) in human subjects and this correlated with reduced adiposity. However, current prebiotic approaches are limited by the steady supplementation with relatively low-complexity molecular structures, which have been shown to promote only a limited set of specialist organisms (Doré and Blottière, 2015).

Mercury and its compounds:

Mercury (Hg) is a toxic metal, which is widely recognized as a global environmental pollutant directly impacting human populations (Erickson and Lin, 2015). Hg is easily distributed in the atmosphere in contrast with other ecological reservoirs, thereby empowering the dispersal and movement of Hg globally (Driscoll et al., 2013). Hg which is released into the atmosphere by

human activities eventually gets deposited into aquatic bodies and terrestrial compartments (Erickson and Lin, 2015) (Fig. 5). After getting deposited, inorganic Hg is transformed with varying efficiencies to methylmercury (MeHg), which may subsequently be taken up by organisms living in close contact with soils and sediments (Erickson and Lin, 2015). Sulfate and iron reducing bacteria are the primary methylators of Hg, and to a lesser extent methanogens, found in the anoxic zone of soils and sediments (Selin, 2009). MeHg is rapidly bioaccumulated and biomagnified in aquatic systems. The utilization of fresh and saltwater fish represents the essential means of MeHg exposure for humans (Driscoll et al., 2013).

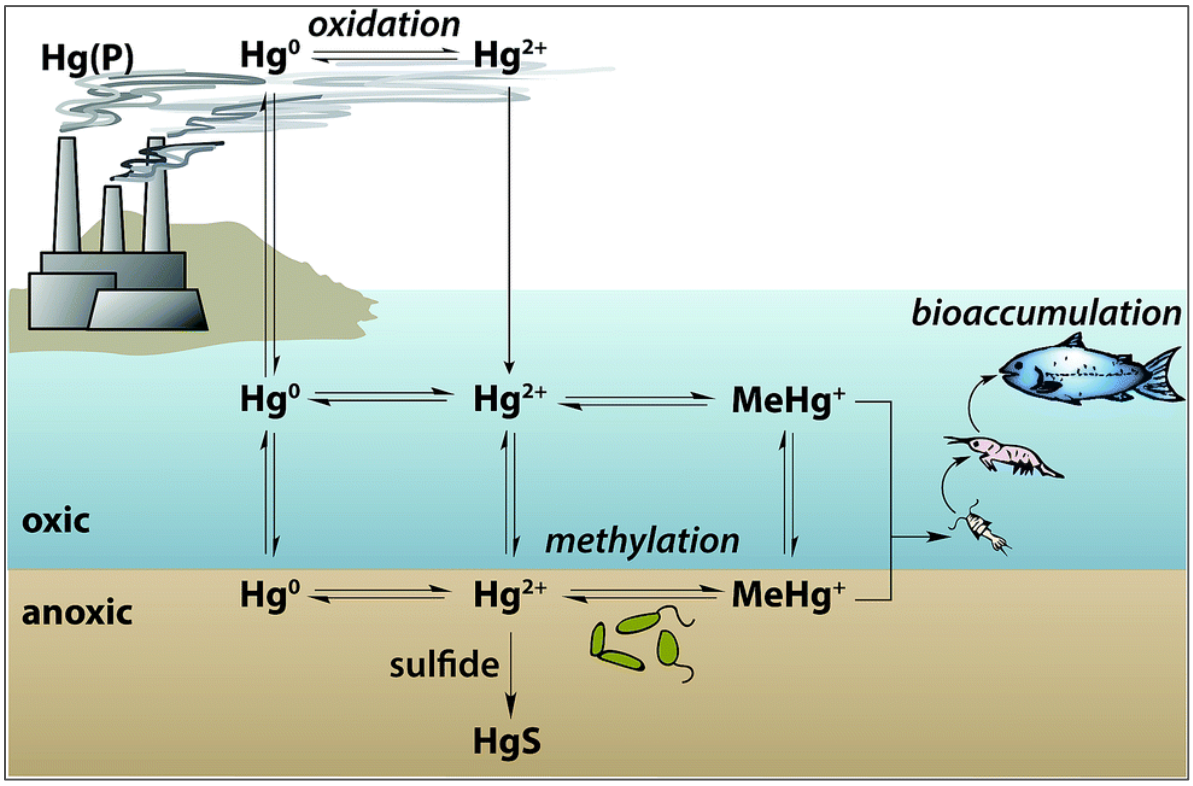

Figure 5: Biogeochemical cycle of mercury. Source: Erickson and Lin, 2015.

Methylmercury toxicity:

MeHg is a potent neurotoxin known to cause severe reproductive and immunological damage to a variety of vertebrates (Boening, 2000). In fish and marine mammals, all the Hg is present mainly in the form of MeHg (Mason et al. 2005). Although humans can get exposed to MeHg in multiple ways (e.g. inhalation, absorption through skin), the main exposition route to MeHg for humans occurs by consumption of fish and marine mammals (Clarkson and Magos, 2006). The estimation of mercury in head hair serves as an indicator of the weight of MeHg in the body and is specifically identified with normal long term fish intake in various populations (Airey, 1983). The intake of MeHg from fish depends on a variety of factors including the average consumption of fish, species of fish and the age of the fish (Clarkson, 1997). Levels of MeHg in edible fish tissue range over at least two orders of magnitude with the highest levels found in predatory fish which are present at the top of the food chain (Clarkson, 1997). The concentration of MeHg (CH_3Hg) has been found of maximum levels in shellfish, particularly piscivorous fish such as tuna (Driscoll et al., 2013). Dietary consumption of marine fish and other seafood is a noteworthy means for introduction of CH_3Hg among human populations, with numerous populations reliant on fish for nourishment, protein, and supplements (Driscoll et al., 2013). The contribution of fish to worldwide diets is expanding (17 kg cap^{-1} yr^{-1} in 2008) (FAO, 2010). In 2007, fish represented 15.7% of the worldwide intake of animal protein which is 6.1% of all protein expended (Driscoll et al., 2013). Worldwide, fish furnishes >1.5 billion individuals with ~20% of their normal per capita intake of animal protein, and 3.0 billion with no less than 15% (Driscoll et al., 2013). China had the most elevated aggregate food fish supply (38.4 million tons; 32.4% of the worldwide supply) and Japan had the most elevated per capita utilization (56.7 kg cap^{-1} yr^{-1} in 2007) (FAS, 2011). The far reaching sullying of fish by CH_3Hg, hence, is a risk to human wellbeing on a worldwide scale (Driscoll et al., 2013).

Developmental neurotoxicity:

The brain and developing central nervous system are the essential focuses for methyl-mercury bringing about marked distal sensory aggravations, choking of visual fields, ataxia, dysarthria, auditory disturbances, and tremors (Harada, 1978; Clarkson and Magos, 2006). This selectivity for the target organs is surprising given the high versatility and reactivity of methyl-mercury in

the body (Clarkson, 1997). MeHg is found in tissues bound to both large (proteins) and small (cysteine and glutathione) sub-atomic weight thiol-containing particles (Clarkson, 1997). The binding of MeHg to glutathione sulhydryl (GSH), because of its high affinity for SH-bunches, diminishes the accessibility of this anti-oxidant factor, rendering the cells vulnerable to free-radical interceded damage (Shanker and Aschner, 2001). MeHg is available as water-soluble complexes and does not distribute in lipid tissues (Clarkson, 1997). It accumulates in the mitochondria (Yoshino et al., 1966) where it diminishes the rate of oxygen utilization, modifies the electron transport chain by impeding complex III (Sone et al., 1977; Yee and Choi, 1996) and instigates loss of the mitochondrial film potential (Bondy and McKee, 1991; InSug et al., 1997; Shenker and Aschner, 2001; Castoldi et al., 2008). The degree of MeHg-instigated cell damage appears to rely upon the intracellular level of antioxidants (Sarafian et al., 1996; Shanker and Aschner, 2001). The binding of MeHg to tubulin and the ensuing aggravations of microtubule assembly/disassembly have been proposed as conceivable components in charge of cytoskeletal adjustments (Graff et al., 1997, Miura and Imura, 1989). MeHg-induced hindrance of the Na^+/K^+ pump and protein synthesis has additionally been observed (Atchison and Hare, 1994). Analyses of human IMR-32 neuroblastoma cells exposed to methylmercury reveal distinguishable modifications in the expression of 7 genes required in signal transduction, transcription (interpretation) and tumor progression (Hwang, 2006). Harada (1978) revealed that at around 6 months of age, 13 of the 220 infants prenatally exposed to MeHg amid the Minamata Bay occurrence suggested the possibility of mercury poisoning as described by instability of the neck, convolutions, and serious neurological and mental disability. Choi et al. (1978) announced anomalous cyto-engineering of the brain in infants prenatally exposed to MeHg.

MeHg enters the placental boundary, achieving more elevated levels in fetal line blood than in maternal blood (Vahter et al., 2000). Studies report that due to active MeHg transport across placental barrier, there is a presence of approximately two fold higher MeHg concentration in fetal blood than maternal (Ha et al., 2017). Despite the fact that it is perceived that the danger of MeHg exposure is more prominent in utero than through breastfeeding, still, maternal milk contains Hg - partly in the Me-Hg form - and therefore may be a source of postnatal infant exposure (Grandjean et al., 1994; Björnberg et al., 2005). MeHg is effectively

exchanged to the fetus over the placenta by means of neutral amino acid transporters during gestation (Kajiwara et al., 1996). Although maternal and cord blood Hg fixation is profoundly associated, cord blood MeHg is reliably higher than the comparing maternal concentration, with a normal proportion of around 1.7 (Mergler et al., 2007; Sakamoto et al., 2004; Stern and Smith, 2003).

Recent research has advanced the understanding of the dose-response relationship between exposure to MeHg and its toxic effects (Driscoll et al., 2013). There are two different ways by which exposure or dose of MeHg can be examined. First, external dose can be assessed by measuring MeHg concentrations in foods, air or in water and multiplying the concentrations by the frequency of exposure (rate of consumption or volume being inhaled over a certain time period). Second, it can be analysed by directly measuring the body burden through measuring the concentrations in tissues such as hair, blood, nails, cord tissues or blood, urine and placenta (Ha et al., 2017). Pregnant women in Minamata had exposure levels 27 times higher than reference areas (Sakamoto et al., 2010), equivalent to 40 μg g^{-1} in hair. In Minamata, fetuses exposed to MeHg through the placenta displayed severe cerebral palsy-like symptoms, while their mothers had mild or no manifestations of poisoning (Driscoll et al., 2013). MeHg were found to cause long-term psychiatric symptoms in adults including impairment of intelligence and mood, and behavioral dysfunction (Yorifuji et al., 2011).

Mechanisms of transport and metabolism of MeHg:

The majority (94-95%) of methylmercury (MeHg) in fish ingested by volunteers was consumed from the gastrointestinal tract (Aberg et al., 1969; Miettinen et al., 1971). Intestinal retention of methylmercury complexed with non-protein sulfhydryl mixes (NPSHs) as happens in bile, was considered to be the methodology for direct infusion of mercury compounds into ligated intestinal segments of rats (Urano et al., 1988). Recent studies document that some specific ingredients of the ingested food substances significantly influence the ability of mercuric ions uptake by enterocytes (Bridges and Zalups, 2017). After retention from the gastrointestinal tract, methylmercury is promptly retained in the blood and is consistently circulated to all tissues, including the erythrocytes, brain and fetus (EPA, 2001). MeHg is actively absorbed by human

erythrocytes through multiple transport mechanisms. However, the most studies report that organic anion transporter is the primary transport system behind the absorption of MeHg by erythrocytes. Additionally, studies also demonstrate the role of Na^{+},K^{+}-ATPase in binding seven molecules of MeHg and $Mg^{+}Ca^{+2}$-ATPase in binding one molecule of MeHg (Bridges and Zalups, 2017). MeHg gets distributed to different portions in the brain but the actual mechanism is not yet fully characterized. But studies suggest that it is possible that neutral amino acid carrier systems present in the luminal membrane of capillary brain endothelial cells are used as transporters for the transport of methylmercury–cysteine complexes. *In vivo* studies in rats and *in vitro* studies in bovine cerebral capillary endothelial cells document that co-administration of Cys followed by MeHg, significantly increase the uptake of MeHg into capillary endothelial cells of blood-brain barrier by forming a MeHg-Cys conjugate. Structural similarities of MeHg-Cys complex and methionine facilitates the transport of MeHg across the blood barrier possibly by an amino acid carrier system called system L (Bridges and Zalups, 2017). Findings on the transportation of MeHg in the placenta are limited but studies suggest that the mechanisms of transportation of MeHg to the placenta tissue are somewhat similar to the transportation to the brain. *In vivo* studies on rats suggest that MeHg is transported to the placenta trophoblasts in the form of transposable species MeHg-Cys conjugates by neutral amino acid carrier called system L in a time and dose-dependent fashion (Bridges and Zalups, 2017).

Contemplates performed with differential centrifugation have demonstrated that methylmercury does not show a particular sub-cellular distribution, since it is available in all fractions (nuclear-, mitochondrial-, lysosomal-, and solvent fraction) (Clarkson, 1972). MeHg in the body is generally steady and is just gradually de-methylated to form mercuric mercury in rats (Norseth and Clarkson, 1970). The demethylation seems to happen in tissue macrophages (Suda and Takahashi, 1986), intestinal microbiota (Nakamura et al., 1977; Rowland et al., 1983) and fetal liver (Suzuki et al., 1984). *In vitro* demethylation has been accounted for to include hydroxyl radicals produced by cytochrome P-450 reductase or hypochlorous corrosive scroungers (Suda and Hirayama, 1992). During the inert period (both amid and after the suspension of introduction), the patient feels no untoward impacts (EPA, 2001). It is conceivable that various biochemical changes may happen in parallel during this period, and some may not

be causatively related with the clinical result (EPA, 2001). Ganther (1978) estimated that the carbon-mercury bond in MeHg experiences homolytic cleavage to discharge methyl free radicals (Me•). The free radicals are relied upon to start a chain of events including peroxidation of lipid constituents of the neuronal cells (EPA, 2001). Studies in mice seem to show that toxicity from exposure to dimethyl-mercury (Me_2Hg) comes about because of the biotransformation of Me_2Hg to MeHg (Ostland, 1969). After intense exposure to MeHg, the majority of the mercury in the brain is in the natural form. In any case, with chronic exposures, a more significant amount is in the inorganic form, indicating that the rate of demethylation increases incrementally with long term exposure (Aschner and Aschner, 1990). Recent critical review by Ha et al. (2017) reported findings of MeHg elimination rates among eight individuals in United States after consumption of 3 fish meals in a two 75 days trials differentiated by a four month wash out period and observed the half-life ranges from 42.5-128.3 days. The study also suggested the variation of ratio of MeHg and inorganic Hg in feces of individuals, providing the evidence that rapid rate of MeHg is associated with a higher percentage of inorganic mercury in feces, an indication of complete de-methylation. Himeno et al. (1989) showed significant enhancement of *in vitro* demethylation activity of MeHg in liver and kidney tissues of seals and rats with long-term feeding of Me-Hg through several generations. Suzuki et al. (1984) and Yamamoto et al. (1986) also reported an enhancement of inorganic mercury formation in normal human fetuses and rats after having been given MeHg for three successive generations. Rice (1989) demonstrated that the tissue half-life of MeHg (75 days) in the brain may be significantly longer than the blood half-life (44 days).

Methylmercury and gut microbiota:

Once ingested, MeHg can undergo biotransformation and be promptly absorbed through the digestive tract. A growing body of scientific proof shows that ingested mercury might be methylated, *in vivo*, in the rat digestive tract (Abdulla et al., 1973) and, *in vitro*, by human defecation (Edwards and McBride, 1975). Studies of Rowland et al. (1973) reported that a major proportion of strains of staphylococci, streptococci, yeasts and *E. coli*, but by only a small percentage of obligate anaerobic bacteria *(Bacteroides* and *Bifidobacteria*) and *Lactobacillus* (isolated from human feces) produced methyl-mercury when grown in pure culture (44 h, pH

7.0) (Table 1). The amount of methylmercury produced by the obligate anaerobes and lactobacilli was much smaller than that by the other bacterial types tested (Rowland et al., 1973). Under anaerobic conditions, the methylation of inorganic mercury in the gut flora is higher than under aerobic conditions (Vonk and Sijpesteijn, 1973). The gut microbiota is additionally ready to demethylate MeHg. As revealed by Clarkson (1972), MeHgCl is changed to inorganic mercury by rat intestinal cells, yet this change happens at a slower rate. Notwithstanding its role in the methylation and the demethylation of Hg, the gut microbiota is fundamental to the disposal of mercury compounds.

Table 1: MeHg synthesis by intestinal bacteria

Micro-organism	**No. tested**	**No. positive**	**Range (ng Me-HgCl formed/ml 44 h)**
Streptococci	6	4	2.1-5.8
Staphylococci	10	6	0.5-5.0
E. coli	5	3	0.9-3.0
Yeasts	9	4	0.7-1.7
Lactobacilli	9	1	0.5
Bacteroides Bifidobacteria	10	3	0.4-0.6

Source: Rowland et al., 1973.

Abdullah et al. (1973) measured the mercury content (total and MeHg) of various tissues after giving 1mg mercuric chloride per day, by gastric incubation for three weeks, to rats with jejunal blind loops and to non-surgically-treated control animals. Retroperistaltic intestinal blind loops are known to harbor a more plentiful microbiota than the normal digestive track (Hillman et al., 2004). In the rats with blind loops, the total mercury and MeHg content, including the brain tissues, were much higher than the control creatures (Hillman, 2004). They, likewise, created manifestations of neurological harm (Hillman, 2004). It appears to be improbable, in any case, that these were induced by MeHg, since these were brain un-weaned mice (Rowland et al.,

1983). These metabolic changes observed during the weaning time frame in mice, harmonize with lactobacilli in a noteworthy modification to the bacterial composition of gut microbiota, thereby diminishing the obligate anaerobes, increasing the Bacteroides (Schaedler, 1973).

Comparing formative changes in demethylating capacity that occur in human gut microbiota, weaned and un-weaned offspring of comparative ages show particularly unique fecal demethylation capacities (Table 2) (Hillman, 2004). The faecal suspensions were incubated with MeHg for 24 h at 37ºC, and % MeHg remaining determined by benzene extraction (Rowland et al., 1983).

Table 2: Effect of age and diet on MeHg demethylation by mouse caecal and human faecal suspensions.

Species	Age	Diet	%MeHg de-methylated
Mouse	10 days	Milk	94
	20 days	Stock	50
	3 months	Stock	46
Man	2 days	Milk	97
	4.5 months	Milk	90
	10 months	Milk	88
	8 months	Solid mixed diet	29
	4.5 years	Solid mixed diet	18

Source: Hillman, 2004.

Genetic basis for mercury methylation:

Studies have demonstrated that Hg is methylated under anoxic conditions (Jensen and Jernelöv, 1969) by sulfate and iron reducing microbes (Compeau and Bartha, 1985; Kerin et al., 2006). Methylation proceeds after cellular uptake of Hg^{II}, methylation of Hg^{II} in the cytosol, and export of CH_3Hg^+ from the cell (Schaefer et al., 2014). Hg methylation is an enzyme catalyzed process proposed to be related with the reductive acetyl–Coenzyme A (CoA) pathway and possibly connected to corrinoid proteins required in this pathway (Choi et al., 1994). Parks et al. (2013) recognized two genes in particular HgcA (encoding a putative corrinoid protein) and HgcB (encoding a 2[4Fe-4S] ferredoxin), required for mercury methylation (Parks et al., 2013). In view of these discoveries, authors propose a mechanical model where a methyl group is exchanged from the methylated HgcA protein to Hg^{II} and the HgcB protein is required for HgcA turnover (Fig. 6) (Poulain and Barkay, 2013). The gene cluster required for methylation is sporadically distributed across two phyla of Bacteria (Proteobacteria and Firmicutes) and one phylum of Archaea (Euryarchaeota) (Parks et al., 2013, Gilmour et al., 2011).

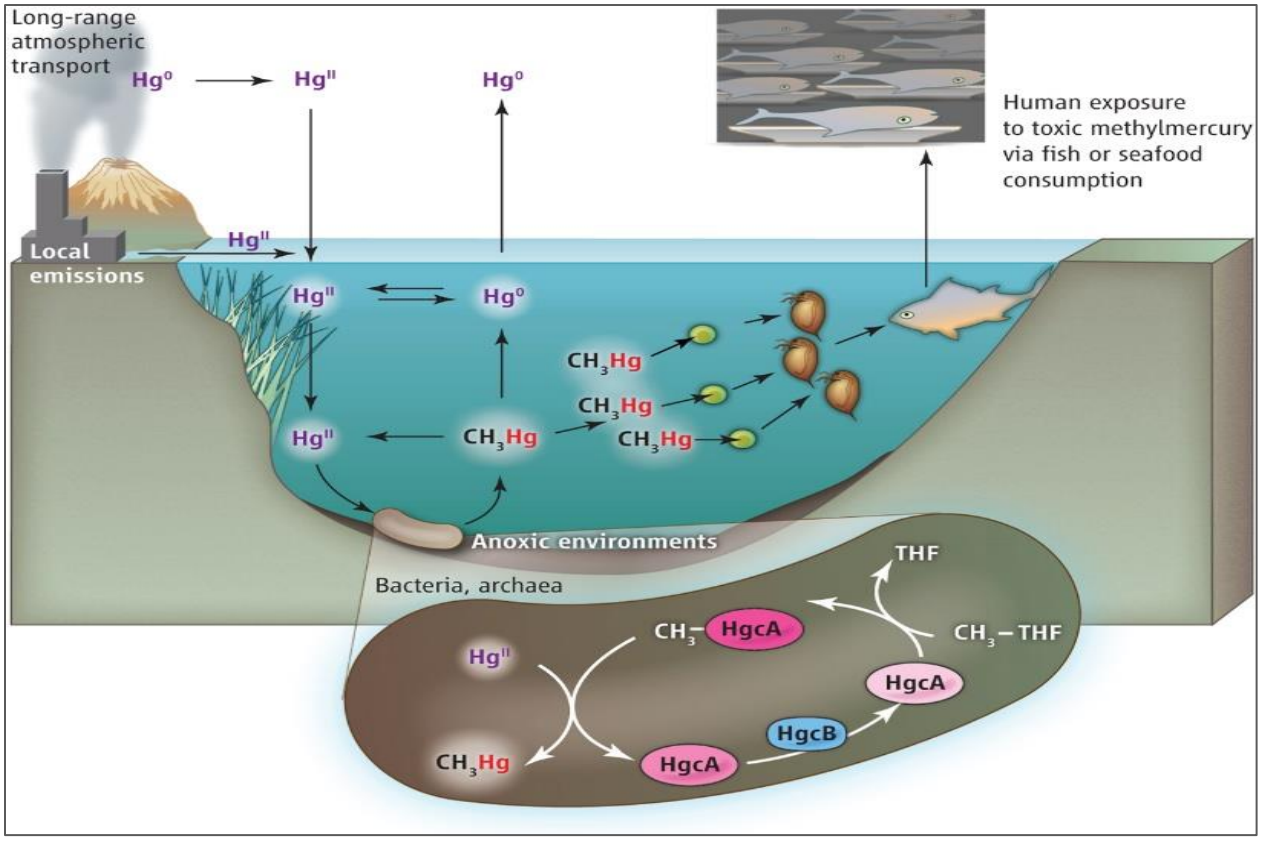

Figure 6: The mercury geochemical cycle. Different colors for the HgcA protein indicate different redox states of the corrinoid HgcA enzyme. THF, tetrahydrofolate.

Source: Poulain and Barkay, 2013.

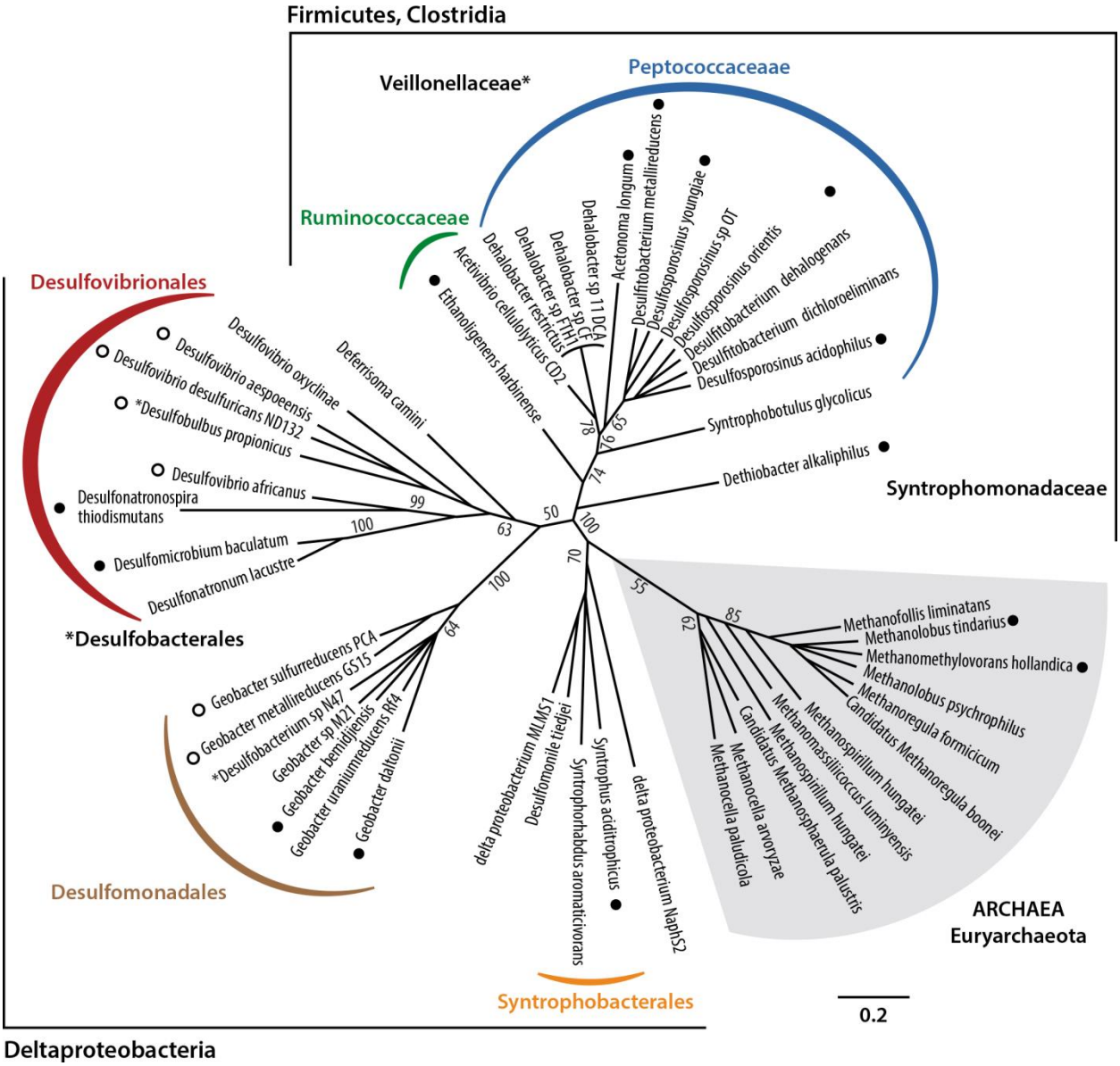

Figure 7: Phylogenetic tree using amino acid sequences from all microorganisms with available genome sequences containing HgcAB orthologues. White dots signify species formerly established as Hg-methylators, black dots show species newly established as Hg-methylators in the study investigated by Gilmour et al., 2013. Source: Gilmour et al., 2013.

Biochemical mechanisms of bacterial resistance to MeHg:

Some microorganisms have evolved a mercury resistance system focused on a gene complex called the *mer*-operon (Clarkson, 1997). An overview of every microbial genome performed in 2010 extended the comprehension of the differences of microorganisms that have the *mer*-operon detoxification machinery and recognized its presence among the early developing genealogies of thermophilic microscopic organisms and Archaea (Lin et al., 2012). This implies that Hg resistance, in all likelihood, originated in a hydrothermal environment, where geochemically determined Hg is at a naturally elevated concentration. The *mer* operon encodes for a homodimeric flavin disulfide oxidoreductase mercuric reductase (MerA), an organomercury lyase (MerB), a periplasmic Hg^{II} scavenging protein (MerP), at least one internal layer of spanning proteins (MerC, MerE, MerF, MerG, MerT) that transport Hg^{II} to the cytoplasm where it is reduced by MerA; some regulatory proteins (MerD, MerR) can also be found in the operon (Fig. 8) (Lin et al., 2012). These genes control the expression of an enzymatic complex involved in Hg scavenging, uptake and transformation (Walsh et al., 1988). The end product of this operon activity is the production of Hg^0, a volatile Hg species that readily leaves the cell via passive diffusion.

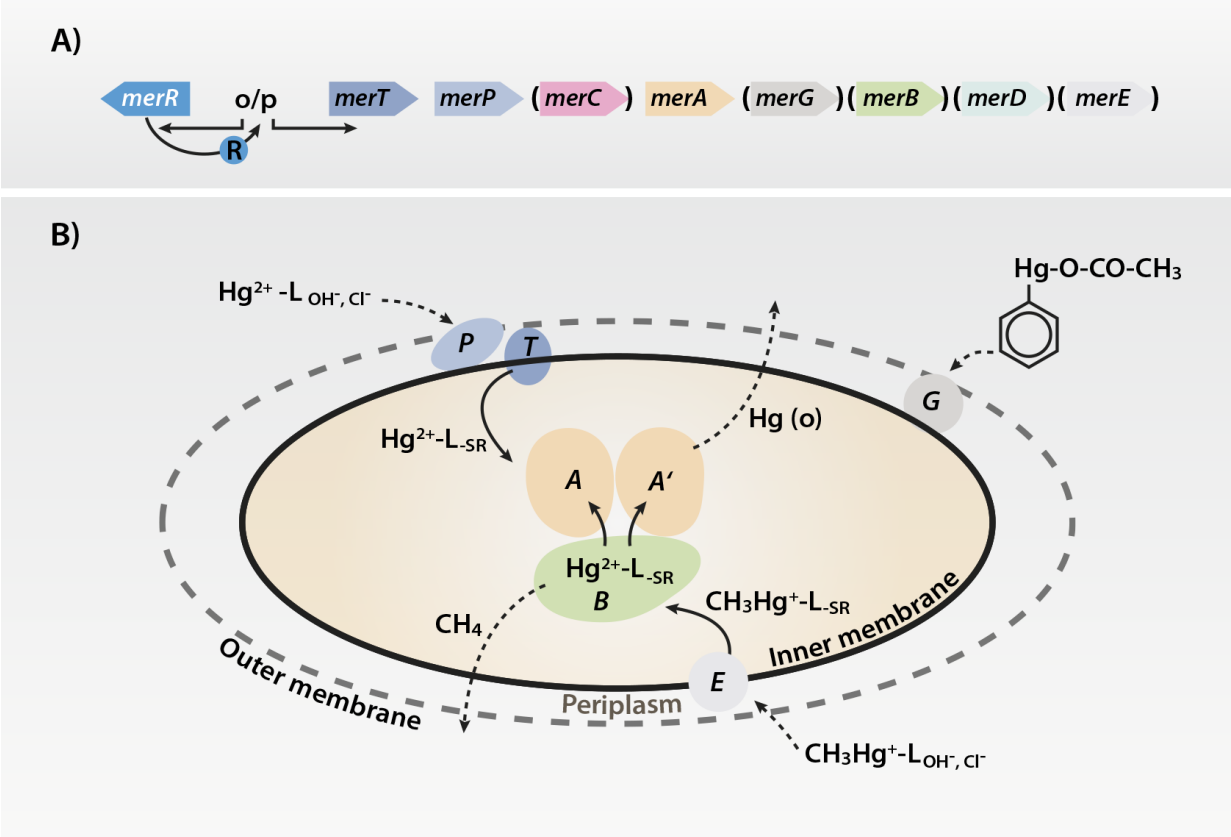

Figure 8: The *mer* system. Source: Lin et al., 2012.

Effect of different diets on Hg metabolism:

Previous studies suggest that dietary components may influence absorption and excretion of Hg compounds. Landry et al. (1979) has shown different diets like chemically defined liquid diet, pelleted rodent diet, and milk diet differentially affected whole body elimination of Hg after MeHg administration. Fecal excretion accounts for greater than 84% of the fecal and urinary Hg excretion of each group during the two-week period (Landry et al., 1979). A milk diet has been reported to enhance gut absorption of certain compounds including lead (Kello and Kostial, 1973) and inorganic Hg (Jugo et al., 1975) by binding of heavy metal compounds to fatty acids from milk triglycerides (Jugo, 1977). The relative content of lipid in the diet (Table 3) is related with the rate of Hg elimination and the relative carbohydrate (Table 3) consumption (Landry et al., 1979). Early studies (Rowland et al., 1975; Greenwood et al., 1977; Landry et al., 1979;

Rowland et al., 1984) demonstrated the ability of the intestinal microflora to demethylate MeHg and its capacity to alter its metabolic activity in response to dietary modifications. This suggested that the diet induced changes in demethylating activity of the gut microbiota. However, a defined diet which could affect Hg metabolism (methylation/demethylation) at the cellular and molecular level has not been established. This work focused on the possible role of different diets in Hg metabolism will further expand our understanding of such processes to control diet-induced mercury toxicity.

Table 3: Major dietary components of three diets.

Diets	Protein Conc	Protein Ingest	Carbohydrate Conc	Carbohydrate Ingest	Lipid Conc	Lipid Ingest
Chemically defined liquid diet	7.2	0.55	31.8	2.43	0.25	0.02
Pellet diet	22.3	0.82	51.5	1.89	5.3	0.19
Evaporated milk diet	6.8	0.56	10.0	0.83	7.56	0.62

Concentrations (conc.) are expressed as g per 100 g of diet (Landry et al., 1979). Average ingestion (ingest) is expressed as g ingested per mouse per day based on measured consumed volumes of the liquid diets (Landry et al., 1979). Source: Landry et al., 1979.

Materials and methods:

Experimental design:

Batch experiment was conducted utilizing small batch fermenters containing collected human feces (serving as source of gut microbiota), spiked mercury isotopes ($^{198}HgCl_2$ and $Me^{199}HgCl$) to examine the potential of human gut microbial species to bio-transform added mercury species over 96 hours' of incubation time period.

Sample collection-Microbiota source:

Freshly voided feces were collected from five healthy controls (all male consuming western diet). Subjects collected samples by placing disposable commode specimen containers (Claflin Medical Equipment, Warwick, RI) under their toilet seats before bowel movements. The five unrelated healthy subjects were free of gastrointestinal disorders and did not use antibiotics prior to 6 months or during the course of the experiment. Fresh fecal samples (n=5 samples/individual) were immediately pooled together and diluted 4:50 in sterile PBS, centrifuged at 5000 rpm for 10 mins and stored at -20ºC before use. Before being introduced into the system, the inoculum was thawed by 1 h immersion in a 37ºC water bath. Because each individual harbors a unique microbial community consisting of complex interactions bacteria, pooling does pose a risk of creating interactions between bacteria that would not occur under physiological conditions (Rose et al., 2009). Nevertheless, pooling has been recommended (McBurney and Thompson, 1989) and utilized by a number of researchers (Fässler et al., 2006; Lebet et al., 1998; Stewart and Slavin, 2006, Hughes et al., 2007) to create a general microbiota that is free of anomalous results that may arise from the microbiota from a single individual (Christl et al., 1997).

***In vitro* lower gastrointestinal fermentation:**

This study utilizes a batch fecal fermentation to estimate the effect of dietary interventions on gut microbial community structure and subsequently its impact on Hg transformations. Although this is a model of what actually occurs in the large bowel (Macfarlane and Macfarlane, 2007), it provides useful data from which to form hypotheses for *in vivo* studies (McBurney and Thompson, 1987). The batch assay consists of serum bottles containing a diet solution (described below in the next section and reference to Table 4), a small intestine solution (described below in the next section) and a microbial solution (using 1 X PBS) extracted from fecal samples for a total of 100 ml in triplicate (Fig. 9). To examine the microbial community Hg transformation potential, Hg and MeHg isotopes were spiked to a duplicate set. 243 µl of MeHg is administered in the form of $Me^{199}HgCl$ from the stock solution containing 4.3 µg/ml to reach a final concentration of 10ng/ml in each vial and 100 µl of inorganic mercury was added in the form $^{198}HgCl_2$ from the stock solution containing 1 µg/ml to attain a final concentration of 100 ng/ml in each vial. All bottling was done under anaerobic conditions using an anaerobic chamber.

Samples are taken out at specific intervals (time 0 and 96 h) for 16s rRNA gene sequencing to characterize the temporal patterns of microbial community structure upon dietary interventions and at intervals of 0, 6, 12, 24, and 48 h to track Hg transformations by inductively coupled plasma - mass spectrometry (ICP-MS).

Substrate used for fermentation: Source and preparation

During the incubation period (96 h), all the batch cultures were fed with simulated human intestinal microbial ecosystem (SHIME) based nutrient solution (57 ml) (Table 4) as described by Laird et al. (2009). The experimental diets used here, high carbohydrate, high protein, and high fat, are modifications of the balanced diet and differed in the carbohydrate, protein and fat content as described in Table 4. The batch cultures were fed with all four different kinds of diets, each representing a nutrient characteristic of western diet (high fat/protein) or traditional diet (high carbohydrate/dietary fiber) acidified to pH 2-3 via the addition of 12 M HCl. Nutrient solution was pumped into the small intestine and immediately mixed with small intestinal solution (30 ml) containing 12.5 g L^{-1} $NaHCO_3$, 6.0 g L^{-1} Oxgall, 0.9 g L^{-1} pancreatin (Laird et al., 2009).

Table 4: Anaerobic batch culture feed- Nutrient solution.

Ingredients	Balanced diet	High carbohydrate	High protein	High fat
$KHCO_3$	5.01	5.01	5.01	5.01
NaCl	2.92	2.92	2.92	2.92
Arabinogalactan	0.5	Twice the amount	0.5	0.5
Pectin	1.0	Twice the amount	1.0	1.0
Xylan	0.5	Twice the amount	0.5	0.5
Potato starch	2.1	Twice the amount	2.1	2.1
Glucose	0.2	Twice the amount	0.2	0.2
Yeast extract	1.5	1.5	Twice the amount	1.5
Peptone	0.5	0.5	Twice the amount	0.5
Mucin	2.0	2.0	2.0	2.0
L-cysteine	0.25	0.25	Twice the amount	0.25
Sodium butyrate	16	16	16	Twice the amount

All concentrations are in gram per litre of Milli-Q^{TM} water.

Figure 9: Set of anaerobic batch culture.

16s rRNA gene amplicon sequencing and processing:

To characterize temporal patterns of microbial community structure, 16s rRNA gene amplicon sequencing was performed on samples collected at certain incubation periods. Samples were kept at -80ºC until DNA was extracted. DNA extractions used a MoBio Power Soil kit (Mo Bio Laboratories, Carlsbad, CA) following the manufacturer's instructions. The V4 region of the 16S rRNA gene was PCR amplified in triplicate using custom barcoded forward primers and the resulting amplicons were checked on a 2% agarose gel to determine the success of amplification and the relative intensity of bands. The amplicons from each sample were equally combined, purified and subjected for quantification and sequencing on the Illumina HiSeq Platform according to the manufacturer's protocols (Mr DNA lab, Shallowater, TX).

The reads were joined after bases with quality scores <25 were trimmed from the ends and reoriented to 5'-3' direction by the sequencing facility (Table 5). All of the further sequence handling was completed with QIIME 1.9.1 (Caporaso et al., 2010) on the HPCVL cluster at Queens University, Canada, unless stated otherwise. For the pipeline used, please refer to the

supplementary data (SD1). Sequences under 300 bp and over 600 bp were removed, the joined reads were demultiplexed, and both forward and reverse primers were removed. Chimeras were removed with vsearch (https://github.com/torognes/vsearch) by matching reads against (unaligned) SILVA123 QIIME release core alignment sequences (Quast et al., 2013). Open reference OTUs (Rideout et al., 2014) were picked with uclust (Edgar, 2010) against the SILVA123 QIIME release 97% clustered sequences in QIIME with prefiltering at 60%, and otherwise using default settings. Singleton, mitochondrial and chloroplast OTUs were removed from the OTU table and a phylogenetic tree was constructed from the sequences with Fast Tree (Price et al., 2010) after aligning them against the SILVA123 QIIME release core alignment using pyNAST 1.2.2 (Caporaso et al., 2010). The OTU table and tree were then exported to R for further processing.

Table 5: Primer sequences for 16srRNA gene amplification and sequencing: (From MR DNA).

Gene	**Forward primer sequence (5'-3')**	**Reverse primer sequence (5'-3')**
16s rRNA	515F GTGCCAGCMGCCGCGGTAA	806R GGACTACHVGGGTWTCTAAT

Bioinformatics and Statistics:

The OTUs that reached at a 97% similarity were used for alpha diversity (Shannon, 1948; Simpson, 1949), and rarefaction curve analysis using a vegan package (Oksanen et al., 2016) in R. Phylogenetic beta diversity measures such as weighted and unweighted UniFrac distance metrics analysis (Lozupone and Knight, 2005) were performed using OTUs for each sample using the QIIME program (Caporaso et al., 2010) and PCoA was conducted using phyloseq package (McMurdie and Holmes, 2013) in R software (R Core Team, 2015). Statistical analyses were performed using R with diversity indices, 2-tailed t-test, one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) in R (R Core Team, 2015) and visualized using GraphPad Prism (Version 7.0). All tests for significance were either one or two-sided, and p values < 0.05 were considered statistically significant.

Determination of total mercury and methylmercury in human fecal samples:

Methylmercury (MeHg) fixations are dictated by capillary gas chromatography combined with nuclear fluorescence spectrometry (GC-AFS) as depicted by Cai et al. (1997). Initial concentrates of fresh fecal specimens are subjected to sodium thiosulfate ($Na_2S_2O_3$) clean up and the organomercury species are isolated as their bromide derivatives by acidic potassium bromide (KBr) and copper sulfate ($CuSO_4$) and consequent extraction into a small volume of dichloromethane (CH_2Cl_2). Total mercury (HgT) was determined by using thermal decomposition, gold amalgamation, and atomic absorption in accordance to EPA 7473. The average concentration (n=3, run in triplicates) of HgT as Hg and MeHg in fecal composites (n=5, weighed and pooled together) is 3.460 µg/kg (w/w) and 0.024 µg/kg (w/w), respectively.

Reagents:

Stock solutions of 4.3 µg/ml $Me^{199}HgCl$ and 1 µg/ml $^{198}HgCl_2$ were used. Hg free deionized water was produced by filtering tap water through a Culligan system consisting of activated charcoal and two mixed bed ion exchange cartridges. The filtered water was piped to a mercury-free clean room, where it was passed through a Barnstead Mega-ohm B Pure system (Cai et al., 1997). KBr, potassium cyanide (KCN), potassium hydroxide (KOH), $CuSO_4$, sulphuric acid (H_2SO_4), $Na_2S_2O_3$, dicholoromethane (CH_2Cl_2), hydrogen chloride (HCl), sodium sulphate (Na_2SO_4) were all obtained from Fisher Scientific.

Extraction of methylmercury:

Spiked samples were taken out from the incubator at various periods as specified above and prepared for sample extraction. 0.5 ml of KOH is added to each specimen and after that each sample is spiked with 100 µl of $Me^{201}HgCl$ as an internal standard for the isotope weakening measurement. Tests are set for homogenization for 30-45 mins at room temperature in the dark followed by 1 h of vigorous blending with a shaker (330 rpm). In the wake of altering pH for all samples at 3.0 with 6 N HCl, 1ml of blend of KBr and $CuSO_4$ (5:1) and 2 ml of CH_2Cl_2 were included quantitatively. All specimens were left for overnight shaking at 330 rpm. Tests were centrifuged at 5,000 rpm for 15 mins to break the emulsion that may have formed. Exact known amount (by weight) of the concentrate was exchanged to clean 7-ml glass vials and 2 ml of

0.0001 M $Na_2S_2O_3$ was added to each sample for refinement. Tests were shaken for 45 mins at 330 rpm, vortexed for 30 s, and afterward centrifuged for 5 mins at 3,000 rpm. Now, two stages could be seen in the vials. Exact known volume from the top aqueous stage ($Na_2S_2O_3$) was then moved into an extraction vial (smaller scale rotator tube), and put away at 4ºC until ethylation and detection.

Analysis:

An appropriate aliquot (0.5-1 ml) of the extract was taken for derivatization by aqueous phase ethylation with sodium tetraethylborate ($NaBEt_4$), and volatile organomercury compounds (e.g. MeHg) were purged and trapped on a Tenax absorber, thermodesorbed onto the GC column, isothermally separated at 105ºC and quantified by ICP-MS as mercury (Hintelmann et al., 1995; Hintelmann and Evans 1997; Hintelmann and Ogrinc, 2003). Chromatographic peak areas of the isotopes (generated by ICP-MS) were used to calculate methylmercury concentrations using a programmed spreadsheet that accounts for procedural blanks and isotope abundance (Hintelmann and Ogrinc, 2003).

Acid extraction of methylmercury:

Samples were prepared (H_2SO_4-KBr-$CuSO_4$ leaching/CH_2Cl_2 extraction) and analyzed (GC-AFS) according to Cai et al. (1997). The procedure generated total methylmercury values of the samples since the MeHg after extraction and $Na_2S_2O_3$ purification underwent solvent extraction (CH_2Cl_2) GC-AFS detection (Avramescu et al., 2010) (Fig. 10).

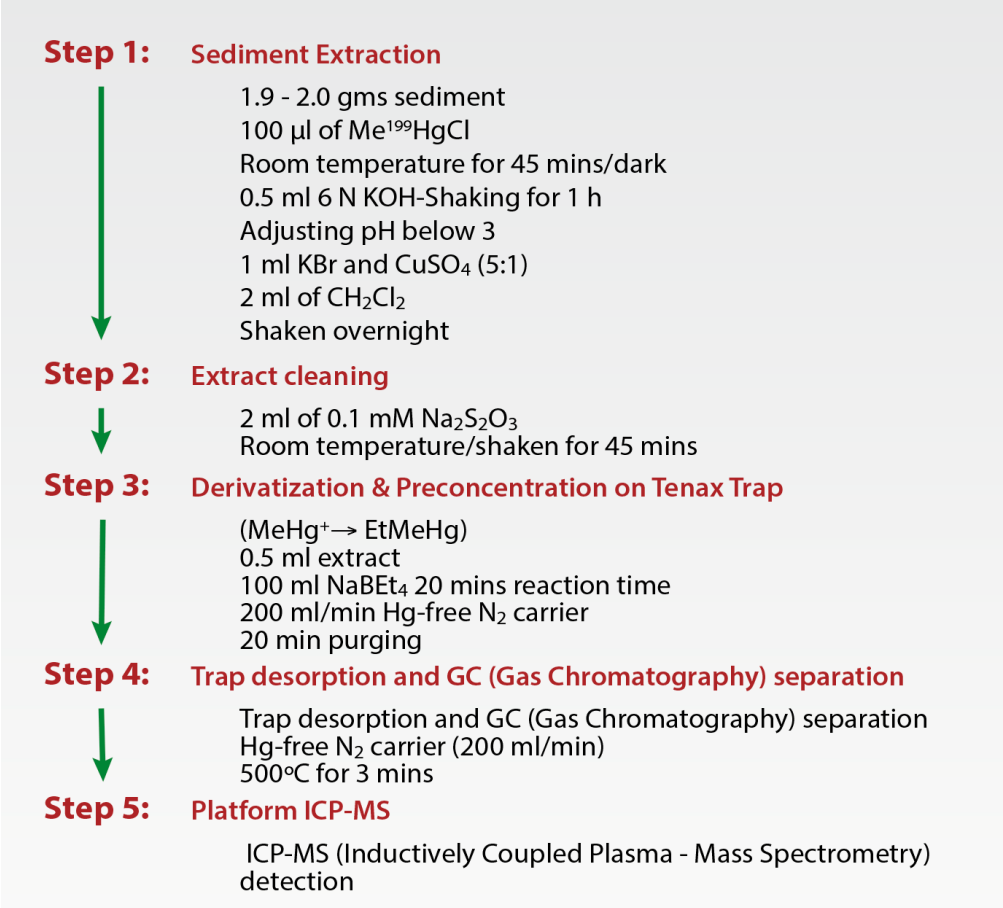

Figure 10: Schematic description of the thiosulfate extraction of MeHg and analysis. Source: Avramescu et al., 2010.

Results

Richness and diversity in the active fecal microbiota across the samples:

After quality control processes filtered out reads containing incorrect primer or barcode sequences and sequences that were <300 nucleotides and >600 nucleotides or with more than one ambiguous base, a total of 1,414,599 sequence reads were obtained. Each individual sample was covered by an average of 88,412 reads (range= 48,217-126,067, SD= 22,666). The total reads, counts and indices of bacterial diversity of OTUs at a 3% dissimilarity level are summarized in supplementary Table S1 (SD1). Fig. 11 indicates the bacterial alpha diversity inter-sample variations from the Shannon diversity index as: T0 carbohydrate> T0 fat> T0 balanced> T0 protein> T0 Hg fat> T0 Hg carbohydrate> T0 Hg protein> T0 Hg balanced> T96

Hg protein> T96 carbohydrate> T96 Hg balanced> T96 fat> T96 protein> T96 balanced> T96 Hg fat> T96 Hg carbohydrate. From Fig. 11, it is clear that there was significant change in the microbial diversity which occurred at time points 0 h and 96 h ($p<0.001$), as calculated by ANOVA (complying with our first hypothesis which was: the microbial community of human gut/batch culture will undergo changes upon dietary interventions within 4 days). From the Shannon diversity index being conducted (Fig. 11), it is evident that there were no significant changes occurred with diet ($p=0.91$; NS>0.05), thus rejecting our predictions (2-5). Furthermore, addition of Hg also did not show any significant effect on microbial diversity ($p=0.06$; NS>0.05).

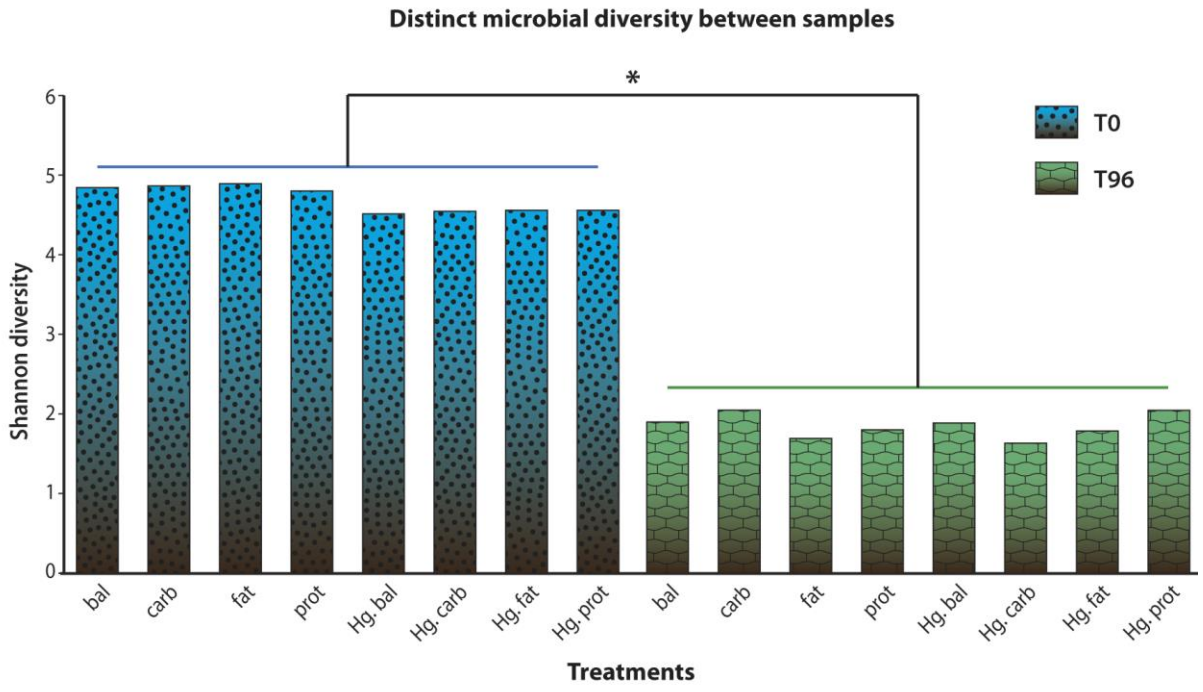

Figure 11: The Shannon index was used to measure the diversity (i.e. a combined assessment of the number of 3% dissimilar bacterial taxa and abundance) across 16 samples. Differences shown as mean with ANOVA. P values are indicated as follows; *P<0.001. T0 and T96 h indicate time points 0 and 96 h, respectively. Bal- balanced diet, carb- carbohydrate diet, fat- fat diet, prot- protein diet, and Hg stands for mercury

The richness of total bacterial communities in samples was analysed by rarefaction analysis over a range of values from minimum 0 to maximum 126,067 reads per sample in the

data. The trend of the rarefaction curves also confirmed the significant differences in richness (as indicated by rarefaction OTU estimates) among samples (Fig. 12) as mentioned above; however, the steep unsaturated shape of the rarefaction curves of T0 bal, carb and fat indicated that the bacterial richness of those samples were not yet sufficiently sampled. OTUs for the rest of the samples (T96) levelled off as the sample sequencing reads increased (Fig. 12), suggesting that sufficient number of sequencing sample reads were accounted to reach a levelled off curve.

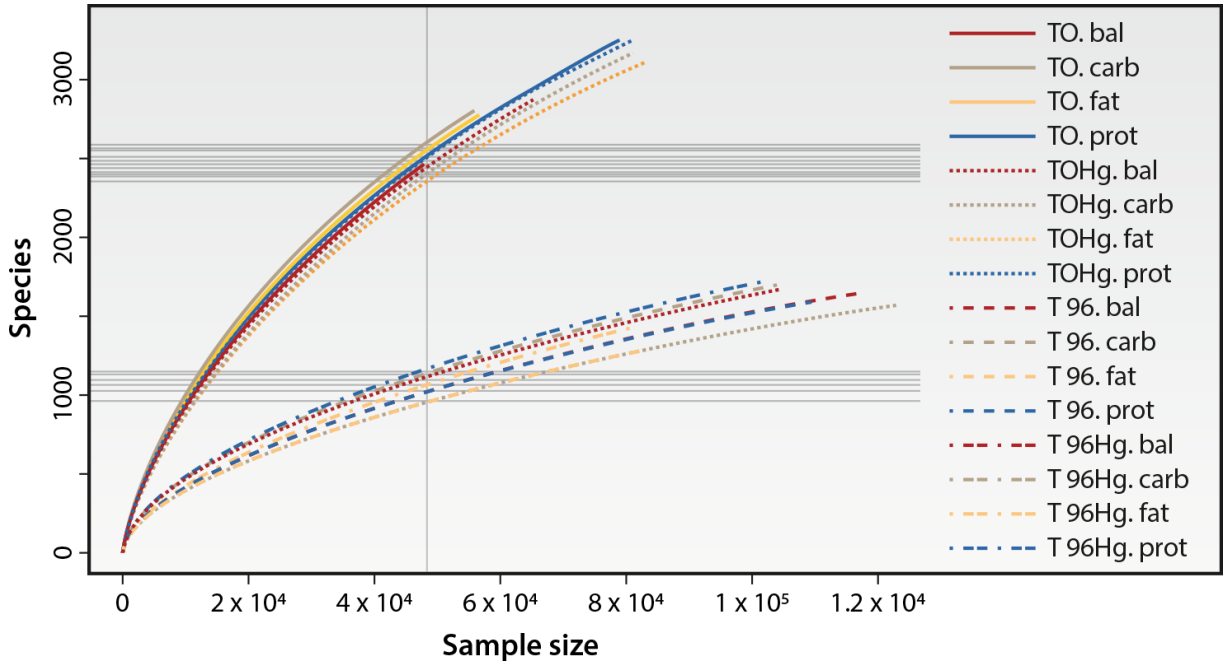

Figure 12: Rarefaction curves were calculated to estimate richness (in this case the number of species at a 3% dissimilarity level) among samples. Repeated samples of OTU subsets were used to evaluate whether further sampling would likely yield additional taxa, as indicated by whether the curve has reached a plateau value. The vertical axis shows the number of OTUs (represented as species here) that would be expected to be found after sampling the number of tags or sequences shown on the horizontal axis. T0 and T96 h indicate two time points 0 and 96 h, respectively. Bal- balanced diet, carb- carbohydrate diet, prot- protein diet, and Hg stands for mercury.

Taxonomy-based comparison of fecal microbiota at the phylum and species levels across the samples:

Gut microbial composition changes over time:

The observed dominant phyla groups were Firmicutes (time = 0 h) and Proteobacteria (time = 96 h). Relative abundance (>1%) of major phyla was measured by statistical analysis incorporating paired t-test among major phylum groups and shown in Fig. 13 and the dominant groups at the family levels are shown in Supplementary Fig. S1. These phyla based OTUs were significantly affected by incubation time and not by short term dietary perturbations (Fig. 13). The abundance of Firmicutes and Proteobacteria phyla accounted for 70% and 85% of all sequences in the samples at time 0 and 96 h, respectively (Fig. 13). While Firmicutes significantly decreased in all diet types over 96 h ($p<0.001$), the relative abundance of Proteobacteria significantly increased ($p<0.001$). T-test based comparisons of phylum level OTUs between Hg treated and non-treated samples indicated no significant differences, although subtle changes in microbial community structure were observed upon Hg spiking (Fig. 13). Addition of Hg to the samples led to negligible reductions in Bacteroidetes in all T0 Hg and T96 Hg fat and T96 protein samples (NS>0.05).

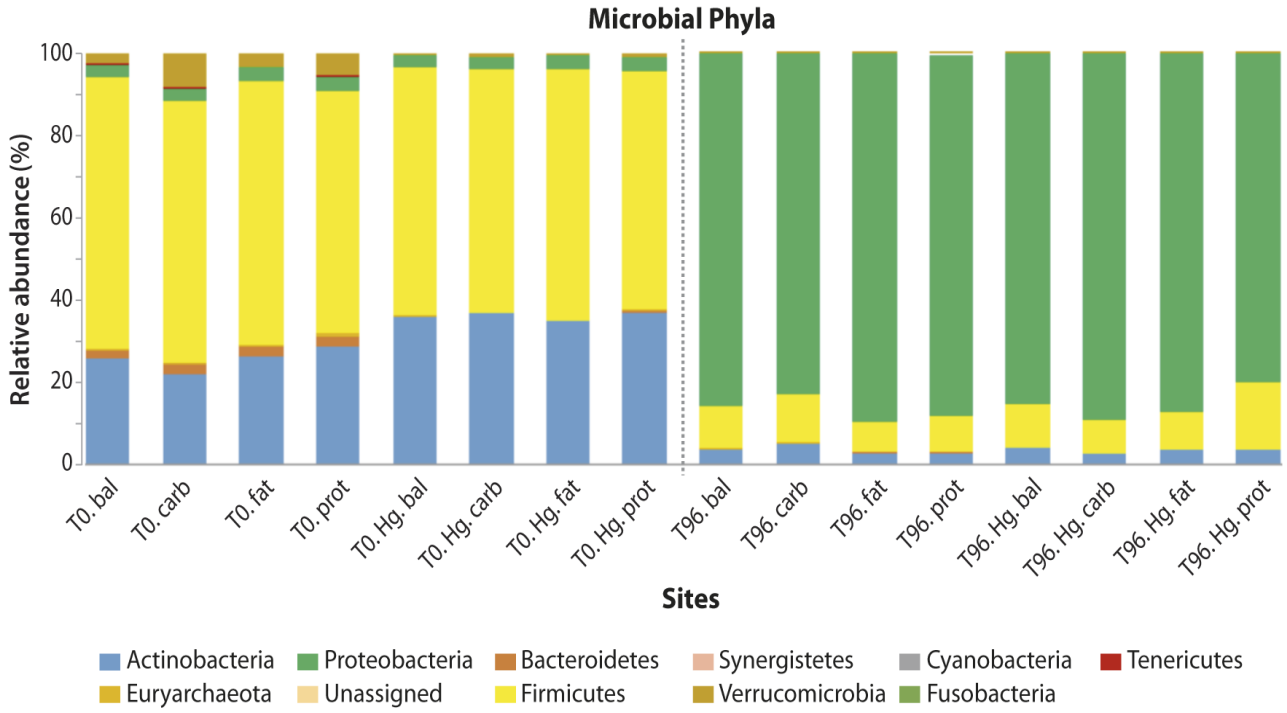

Figure 13: Changes in the relative abundance of bacterial communities in the samples at the phylum level at time 0 (T0) and 96 h (T96). Data shown as mean of paired t-test. Bal- balanced diet, carb- carbohydrate diet, prot- protein diet, and Hg stands for mercury.

To visualise the time-dependent response of the gut microbiota, we performed a UniFrac analysis, an algorithm that measures similarity among microbial communities based on the degree to which their component taxa share branch length on a bacterial tree of life (Luzopone and Knight, 2005). All pair-wise distances between communities were computed and PCoA was used to cluster the communities' axes of maximal variance. Both weighted and unweighted UniFrac analysis showed a similar pattern change of the fecal microbiota in response to time, with a greater degree of segregation by weighted UniFrac analysis compared to the unweighted one (Fig. 14 and Fig. 15). A relatively large distance between the two sets of samples (T0 and T96) indicated a quite different structure of microbiota in the samples. The gut microbial community of each sample was clustered by time points and the maximum variations were 96% (PC1) and 3.2% (PC2) in weighted and 34.5% (PC1) and 6.4% (PC2). MANOVA and ANOVA

(followed by Tukey's post-hoc test) based on the weighted UniFrac (Fig. 14) and unweighted UniFrac (Fig. 15) also explains significant clustering of the samples by time ($p<0.001$), indicating distinct community composition and structure. After 96 h, the microbial community structure was overwhelmingly dominated by Enterobacteria (e.g., *E. coli*). Although *E. coli* are expected to be present as part of the microbiota of a healthy individual, the abundance encountered here is unusual. This may reflect an issue with the incubation protocol or some uncontrolled characteristic of the inoculum.

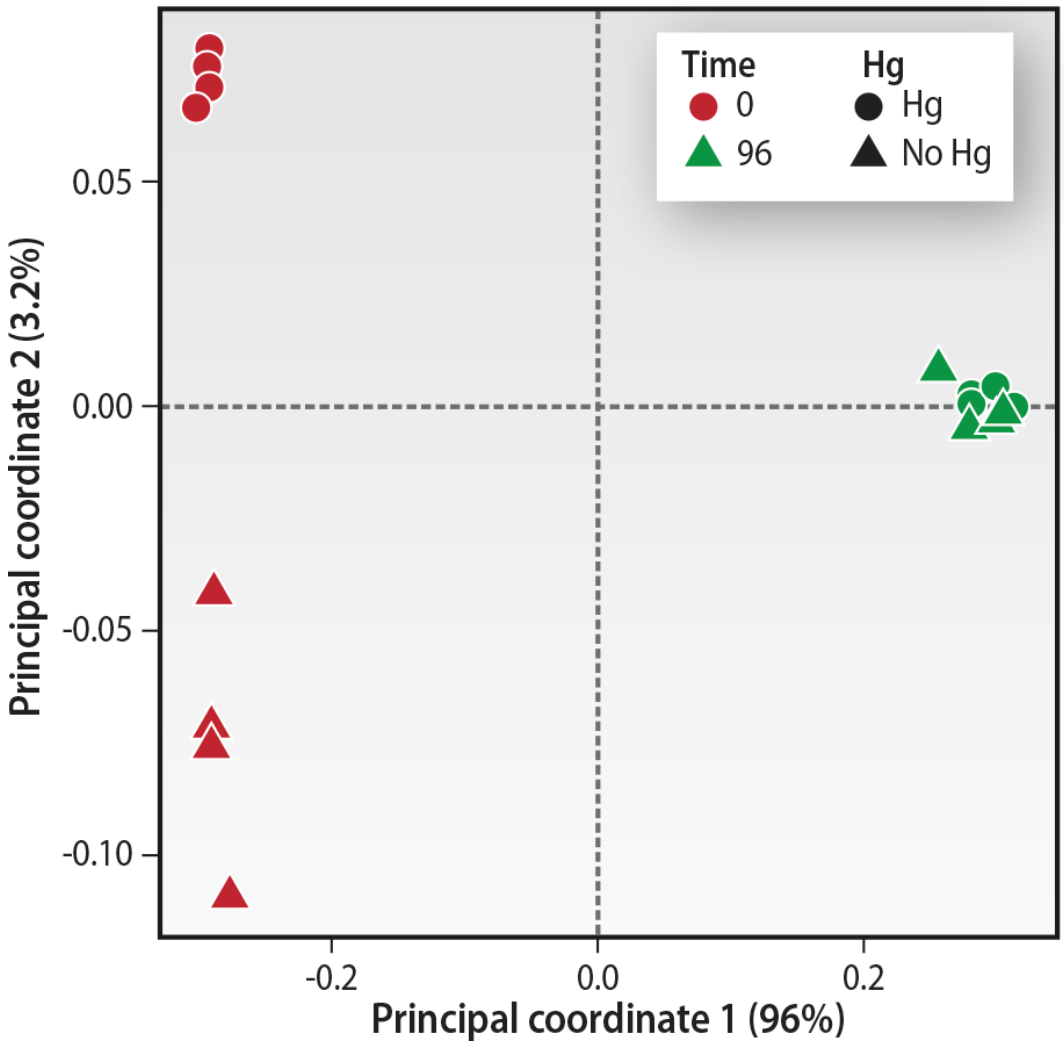

Figure 14: PCoA score plot based on **weighted UniFrac metric**. Clustering of the group means based on the Mahalanobis distance calculated by using MANOVA and ANOVA (followed by Tukey's post hoc test) on weighted UniFrac PCoA. P value indicated as $p<0.001$ along PC 1 and NS>0.05 ($p=0.98$) along PC 2. T0 and T96 h indicate two time points 0 and 96 h, respectively and Hg stands for mercury.

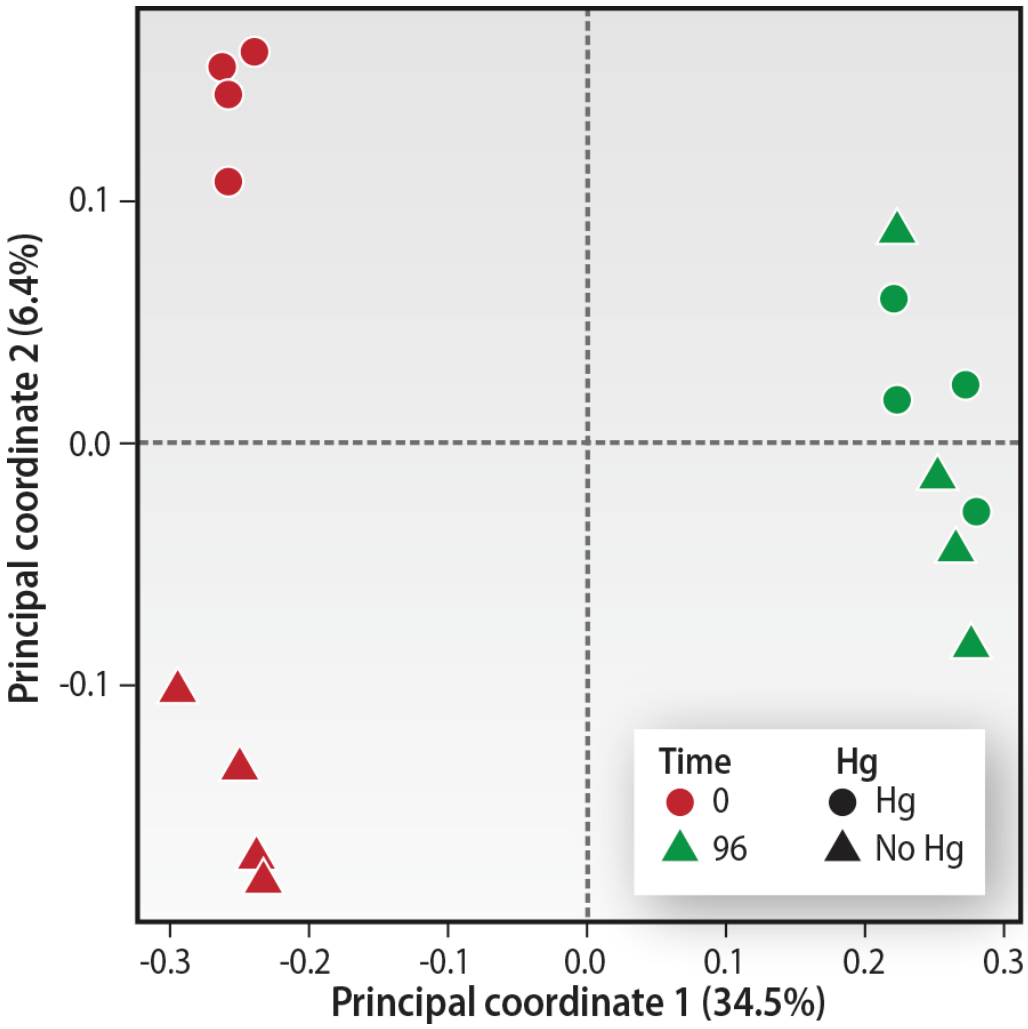

Figure 15: PCoA score plot based on **unweighted UniFrac** metric. Clustering of the group means based on the Mahalanobis distance calculated by using MANOVA and ANOVA (followed by Tukey's post hoc test) on unweighted UniFrac PCoA. P value indicated as $p<0.001$ along PC 1 and NS>0.05 ($p=0.975$) along PC 2. T0 and T96 h indicate two time points 0 and 96 h, respectively and Hg stands for mercury.

Association between gut microbiota and Hg transformation:

Having defined the compositional and temporal variations in the active gut microbiota, we sought to determine the response of this distinctive subset to mercury biotransformation. Figs. 16 and 17 demonstrate the production of MeHg over time at different initial concentrations of

spiked $Me^{199}HgCl$ and $Hg^{198}Cl_2$. The production of $Me^{199}Hg$ increased significantly ($P<0.001$) while there was no increase in demethylation ($p=0.97$; NS>0.05). The production of MeHg was small in all treatments and no significant differences were found. The lack of significant MeHg production was likely due to the overwhelming presence of Enterobacteria which known to be poor methylators. The simultaneous determination of $Me^{199}HgCl$ and $Hg^{198}Cl_2$ using stable isotopes allowed demonstration of considerable differences in the production of these two mercury species. Over the incubation time, the amounts of $Me^{199}HgCl$ formed was significantly ($P<0.001$) higher than those of inorganic mercury; maximum levels of $Me^{199}HgCl$ formation were produced during 30-48 h (consistent with the results of Edwards and McBride, 1975) in all dietary groups (Fig. 16), where the growth of δ-Proteobacteria was maximal, thereby coinciding with results of Edwards and McBride (1975), and Gilmour et al. (2013), respectively. This phenomenon could be due to anaerobic fermentation which created a neutral pH (6.5-7.2) in the medium favouring growth of fermentative Firmicutes, a potent SRB of the genus *Delsulfovibrio* in the class δ-Proteobacteria, known to methylate mercury. However in this study, the proportional abundance of the genus *Desulfovibrio* during first few hours of incubation was too low to be detected (0.009%) and eventually dying out at T96. Hence, we hypothesize that the small number of *D. desulfuricans* together with unclassified members of δ-Proteobacteria during log phase growth, contribute to the formation of very small amounts of methylmercury in the samples. *D. desulfuricans* isolated from the human gut are known to carry genes (*Hgc*AB) essential for mercury methylation.

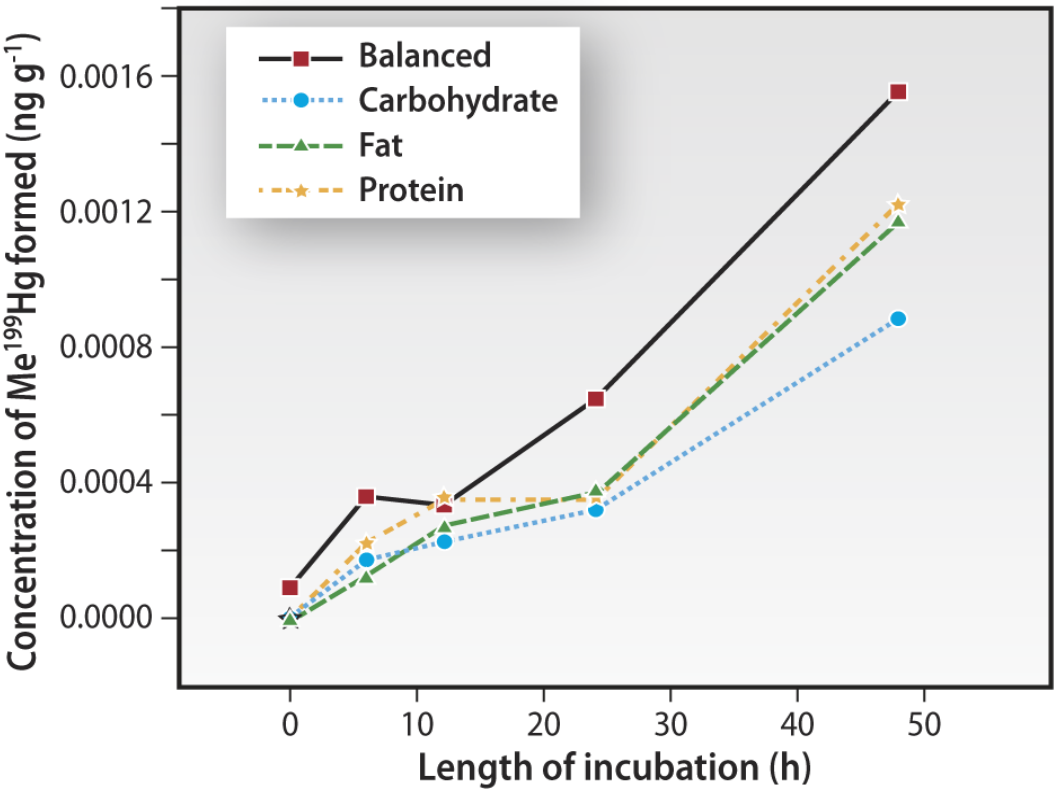

Figure 16: Rate of methylation - Amounts of $Me^{199}Hg$ significantly increased ($P<0.001$) in all dietary groups through time in batch culture.

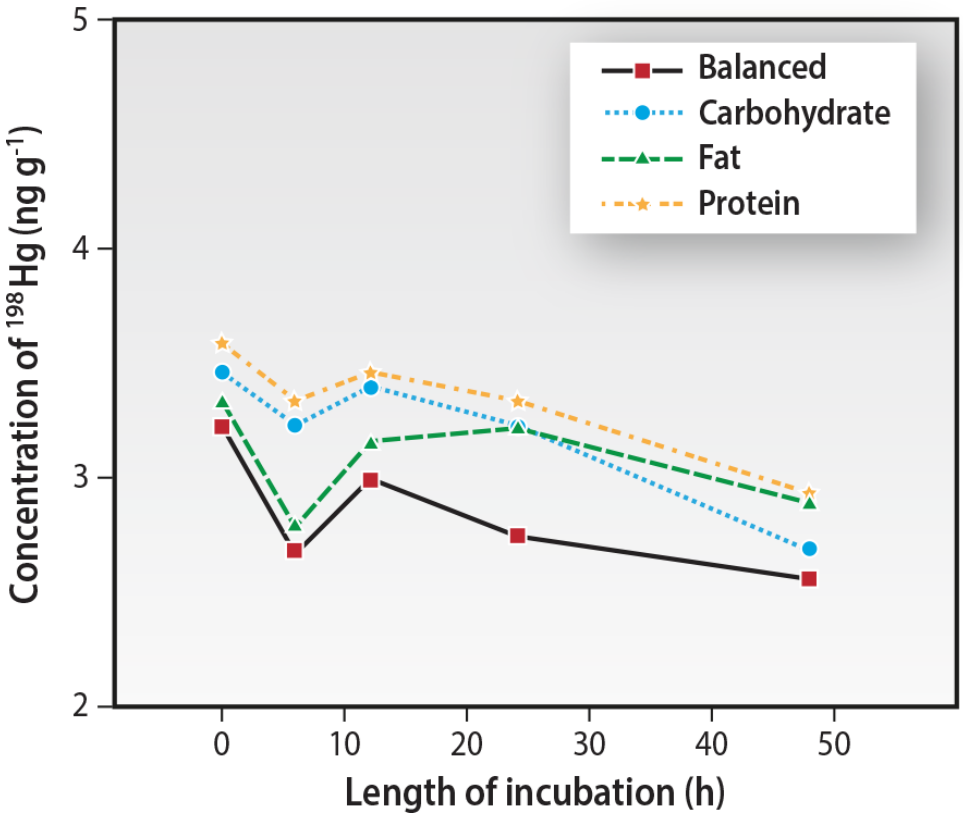

Figure 17: Rate of demethylation- No significant changes in demethylation observed through time.

Discussion:

Our study findings do not support our predictions (1-5) as the diversity of intestinal microbiota significantly decreased over incubation time (Fig. 11), showing no response to different dietary exposure. Weighted (Fig. 14) and unweighted UniFrac (Fig. 15) showed a significant time induced gut microbial variations where samples in t0 group cluster together and away from those in the t96 group representing an inter-sample variability and dissimilarity. We also observed a non-significant increase of *in vitro* methylation (Fig. 16) using $Hg^{198}Cl_2$ tracer. In contrast, no $Me^{199}HgCl$ degradation (Fig. 17) was observed over the incubation period suggesting that diet might have played a role in suppressing the growth of demethylators (SRB, methanogens) in the

samples. The decreased in microbial diversity over time (T96) was found to be overly dominated by one OTU associated with *E.coli* under the phyla Proteobacteria, the most abundant species in this study. This high prevalence of *E.coli* in human gut microbiota is unusual as Proteobacteria only constitutes only 9% of the human gut (Arumugum et al., 2011). *E.coli* strain K12 was found to be most abundant species in this study which is usually a rare phyla colonizing human gut microbiota. Addition of Hg in the fecal pooled cultures seemed to have no effect on microbial diversity over time, thus no modulation in mercury biotransformation was observed over incubation time (thus rejection our prediction 6). Occurrence of insignificant methylation over incubation time is associated low amounts of SRB population (0.009%) in the incubated culture samples. The absence of demethylation over incubation period could be associated with low number of demethylators carrying *merB* gene (organomercuric lyase) responsible for mercury detoxification. The over-dominance of *E.coli* strain in the incubation culture samples might have suppressed the growth of other potential microorganisms (carrying *merB* gene) responsible for mercury detoxification in the human gut.

Our findings are not in agreement with the recent study conducted by Aguiree et al. (2015) where they showed different modulations of gut microbiota by carbohydrate and protein diets after 24 h of incubation, though the differences became more accentuated over time (72 h). In the present study, the level of microbial diversity in different dietary samples was not distinguishable and is considerably lower than that reported in previous studies. For example, Aguiree et al. (2015) reported high levels of FAFV (Firmicutes, Actinobacteria, Fusobacteria and Verrucomicrobia) phyla in a carbohydrate rich diet. Aguirre et al. (2015), Maier et al. (1974) and Zimmer et al. (2012) reported high abundance of certain Bacteroidetes phylum, including *Bacteroides ovatus* and *Bacteroides fragilis* in a high protein diet. Human gut microbiota exhibits plasticity, but in our present study the plasticity of the gut microbiota in response to dietary changes was not adaptive enough to bring a change in gut microbial diversity. Considering the remarkable resilience of the gut community to short term perturbation, it is likely that microbiota adaptation is largely reversible on short time scales (Dethlefsen and Relman, 2010; Lee et al., 2013; David et al., 2014; Aguirre et al., 2015). However, here the ability of the microbiota to accommodate short-term dietary change may be very slow or could be deleterious to its own maintenance. It is possible that the microbiota has not been able to keep

pace with the profound changes brought on by dietary constituents in such a small scale to produce marked inter-diet differences in the microbiota. The smaller incubation vessels, dietary constituents used in our study compared to theirs (Lee et al., 2013; Aguirre et al., 2015) might have influenced the fact that we were not able to observe any significant differences over a longer time scale of 96 h. Vitamins like nicotinamide (Vit B3) and biotin (Vit B7) which are essential for bacterial growth/survival (as Nicotinamide is an important coenzyme in electron transport chain and biotin serves as a cofactor in central cellular metabolism pathways) were not administered in the dietary system; that might have affected the diet-dependent response of microbial diversity. Moreover, the GI *in vitro* model used in the present study was static mono-compartmental, with a limited number of simulated parameters (realistic transit time, pH and enzymatic conditions) available. None of the static models provide an accurate estimation of the *in vivo* condition; reproduce dynamic processes occurring during human digestion such as gastric emptying or continuous changes in pH and secretion flow rates (Guerra et al., 2012). We speculate this might have also affected the changes in the diversity and abundance of intestinal microbiota. This study provides some insights into the structure of the human microbial ecosystem but still some significant questions remain unanswered regarding: 1) the potential of certain dietary compounds to promote a specific microbial composition, and 2) the role of certain bacteria in Hg transformations. Detectable differences following a diet change in humans have not been fully explored, and it is unclear how fast and how reproducibly the microbiota can respond to these changes (Muegge et al., 2011; David et al., 2014). One of the few studies in this area was performed by David et al. (2014), who clearly demonstrated that consumption of a diet mainly composed of either plant or animal products modifies the composition and activity of the gut microbiota of healthy subjects in only five days. The authors found that, already one day after initiating the study, there was an increase in β-diversity in microbiota from feces of individuals consuming the animal diet and that their original structure was recovered two days after finishing the study (Aguirre et al., 2015). Our results explain a short-term time dependent response of the gut microbiota after being exposed to different diets, providing the basis for follow up studies which will not only provide novel insight to host-microbial interactions, but may also provide a rich understanding of the unexplained diet-dependent gut microbial changes. Since no variations in methylation and demethylation were observed among dietary groups, our results are not necessarily applicable to populations for toxicological risk assessment. An in-

depth evaluation should be made of the influence of diet on MeHg transformations in the human gut. This kind of study would provide reliable data that could serve as a basis for risk evaluations elucidating the effects of dietary contents on MeHg metabolism.

Significance of Research:

Gastric and small intestinal (GSI) models are increasingly used as an alternative to *in vivo* assays to answer many questions raised by industry and researchers (Guerra et al., 2012). A broad range of *in vitro* systems is available, from static mono-compartmental to dynamic multi-compartmental models. However, these models require a compromise between technological complexity and biological significance (Guerra et al., 2012). In our laboratory, a batch fermentation system was developed to simulate the microbial ecosystem of the small and large intestine. With regard to the results observed and results that have been predicted, the batch reactors we used can be considered as a preliminary tool for studying the microbial composition and activities in the human intestinal tract (Molly et al., 1993). Due to the absence of absorption of metabolites and fluid, the value of the simulator is limited to the study of the principal interactions in the microbial community. Yet, because a variety of additives can easily be administered (e.g. bile salts, bile-salt-hydrolysing enzymes, probiotics, drugs, prodrugs) the batch reactors can be used to evaluate the principal influences of the latter on the ecology of human gut bacteria (Molly et al., 1993). Scientific and technological efforts need to be pursued to improve artificial digestive systems further, extend their potential and approach the *in vivo* human situation still more closely (Guerra et al., 2012).

References:

Abdulla M, Arnesjö B, & Ihse I. (1973). Methylation of inorganic mercury in experimental jejunal blind-loop. *Scandinavian Journal of Gastroenterology* **8**(6): 565–567.

Aberg B, Ekman L, Falk R, Greitz U, Persson G, & Snihs J. (1969). Metabolism of methyl mercury (Hg) compounds in man: Excretion and distribution. *Achieves of Environmental Health* **19**(4): 478–484.

Afonne OJ, Orisakwe OE, Obi E, Dioka CE, & Ndubuka GI. (2002). Nephrotoxic actions of low-dose mercury in mice: Protection by zinc. *Archives of Environmental Health* **57**(2): 98–102.

AGNS (2007): Food Quality and Standards Service (AGNS). Technical report on prebiotics. FAO; 15–16 September 2007; Rome, Italy.
Doi: http://www.fao.org/ag/agn/agns/index_en.stm.

Aguirre M, Eck A, Koenen ME, Savelkoul PH, Budding AE, & Venema K. (2015). Diet drives quick changes in the metabolic activity and composition of human gut microbiota in a validated in vitro gut model. *Research in Microbiology* **167**(2): 114–125.

Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, Gaskins HR, Stumpf RM, Yildirim S, Torralba M, Gillis M, Wilson BA, Nelson KE, White BA, & Leigh SR. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *ISME Journal* **7**(7): 1344–1353.

Amato KR, Yeoman CJ, Cerda G, Schmitt CA, Cramer JD, Miller ME, Gomez A, Turner TR, Wilson BA, Stumpf RM, Nelson KE, White BA, Knight R, & Leigh SR. (2015). Variable responses of human and non-human primate gut microbiomes to a Western diet. *Microbiome* **3**: 53 (9 pp).

Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, … & Bork P. (2011). Enterotypes of the human gut microbiome. *Nature* **473**(7346): 174–180.

Aschner M, & Aschner JL. (1990). Mercury neurotoxicity: Mechanisms of blood-brain barrier transport. *Neuroscience & Biobehavioral Reviews* **14**(2): 169–176.

Atchison WD, & Hare MF. (1994). Mechanisms of methylmercury-induced neurotoxicity. *FASEB Journal* **8**(9): 622–629.

Avramescu ML, Zhu J, Yumvihoze E, Hintelmann H, Fortin D, & Lean DR. (2010). Simplified sample preparation procedure for measuring isotope-enriched methylmercury by gas chromatography and inductively coupled plasma mass spectrometry. *Environmental Toxicology and Chemistry* **29**(6): 1256–1262.

Barcenilla A, Pryde SE, Martin JC, Duncan SH, Stewart CS, Henderson C, & Flint HJ. (2000). Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Applied and Environmental Microbiology* **66**(4): 1654–1661.

Barkay T, Miller SM, & Summers AO. (2003). Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiology Reviews* **27**(2-3): 355–384.

Bazzocco S, Mattila I, Guyot S, Renard CM, & Aura AM. (2008). Factors affecting the conversion of apple polyphenols to phenolic acids and fruit matrix to short-chain fatty acids by human faecal microbiota in vitro. *European Journal of Nutrition* **47**(8): 442–452.

Betian HG, Linehan BA, Bryant MP, & Holdeman LV. (1977). Isolation of a cellulolytic Bacteroides sp. from human feces. *Applied and Environmental Microbiology* **33**(4): 1009–1010.

Björnberg KA, Vahter M, Berglund B, Niklasson B, Blennow M, & Sandborgh-Englund G. (2005). Transport of methylmercury and inorganic mercury to the fetus and breast-fed infant. *Environmental Health Perspectives* **113**(10): 1381–1385.

Björnberg KA, Vahter M, Grawé KP, & Berglund M. (2005). Methyl mercury exposure in Swedish women with high fish consumption. *Science of the Total Environment* **341**(1-3): 45–52.

Boening DW. (2000). Ecological effects, transport, and fate of mercury: A general review. *Chemosphere* **40**(12): 1335–1351.

Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Org E, Parks B, Lusis AJ, Knight R, Caporaso JG, & Svanbäck R. (2014). Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nature Communications* **5**: 4500 (13 pp).

Bondy SC, & McKee M. (1991). Disruption of the potential across the synaptosoma plasma membrane and mitochondria by neurotoxic agents. *Toxicology Letters* **58**(1): 13–21.

Booijink CC, Boekhorst J, Zoetendal EG, Smidt H, Kleerebezem M, & de Vos WM. (2010). Metatransccriptome analysis of the human fecal microbiota reveals subject-specific expression profiles, with genes encoding proteins involved in carbohydrate metabolism being dominantly expressed. *Applied and Environmental Microbiology* **76**(16): 5533–5540.

Booijink CC, El-Aidy S, Rajilić-Stojanović M, Heilig HG, Troost FJ, Smidt H, Kleerebezem M, De Vos WM, & Zoetendal EG. (2010). High temporal and inter-individual variation detected in the human ileal microbiota. *Environmental Microbiology* **12**(12): 3213–3227.

Borenstein E. (2012). Computational systems biology and in silico modeling of the human microbiome. *Briefings in Bioinformatics* **13**(6): 769–780.

Bridges CC, & Zalups RK. (2017). Mechanisms involved in the transport of mercuric ions in target tissues. *Archives of Toxicology* **91**(1): 63–81.

Buddington RK, Williams CH, Chen SC, & Witherly SA. (1996). Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. *American Journal of Clinical Nutrition* **63**(5): 709–716.

Cai Y, Tang G, Jaffé R, & Jones R. (1997). Evaluation of some isolation methods for organomercury determination in soil and fish samples by capillary gas chromatography–atomic fluorescence spectrometry. *International Journal of Environmental Analytical Chemistry* **68**(3): 331–345.

Calabrese EJ. (1978). Nutrition and Environmental Health. Wiley, New York, NY.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, … & Knight R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**(5): 335–336.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, & Knight R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Science USA* **108**(Suppl 1): 4516–4522.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, … & Knight R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal* **6**(8): 1621–1624.

Carvalho-Wells AL, Helmolz K, Nodet C, Molzer C, Leonard C, McKevith B, … & Tuohy KM. (2010). Determination of the in vivo prebiotic potential of a maize-based whole grain breakfast cereal: A human feeding study. *British Journal of Nutrition* **104**(9): 1353–1356.

Castoldi, AF, Johansson C, Onishchenko N, Coccini T, Roda E, Vahter M, ... & Manzo L. (2008). Human developmental neurotoxicity of methylmercury: Impact of variables and risk modifiers. *Regulatory Toxicology and Pharmacology* **51**(2): 201–214.

Chapman L, & Chan HM. (2000). The influence of nutrition on methyl mercury intoxication. *Environmental Health Perspectives* **108**(Suppl 1): 29–56.

Chassard C, Delmas E, Robert C, & Bernalier-Donadille A. (2010). The cellulose-degrading microbial community of the human gut varies according to the presence or absence of methanogens. *FEMS Microbiology Ecology* **74**(1): 205–213.

Choi CM, Lapham LW, Amin-Zake L, & Saleem T. (1978). Abnormal neuronal migration, deranged cerebral cortical organization and diffuse white matter astrocytosis of human fetal brain: A major effect of methylmercury poisoning in utero. *Journal of Neuropathology and Experimental Neurology* **37**(6): 719–732.

Choi SC, Chase Jr. T, & Bartha R. (1994). Enzymatic catalysis of mercury methylation by Desulfovibrio desulfuricans LS. *Applied and Environmental Microbiology* **60**(4): 1342–1346.

Christl SU, Bartram HP, Paul A, Kelber E, Scheppach W & Kasper H. (1997). Bile acid metabolism by colonic bacteria in continuous culture: Effects of starch and pH. *Annals of Nutrition and Metabolism* **41**(1): 45–51.

Clarkson TW. (1972). The pharmacology of mercury compounds. *Annual Review of Pharmacology* **12**(1): 375–406.

Clarkson TW. (1997). The toxicology of mercury. *Critical Reviews in Clinical Laboratory Sciences* **34**(4): 369–403.

Clarkson TW, & Magos L. (2006). The toxicology of mercury and its chemical compounds. *Critical Reviews in Toxicology* **36**(8): 609–662.

Cole JR, Chai B, Farris RJ, Wang Q, Kulam SA, McGarrell DM, Garrity GM, & Tiedje JM. (2005). The Ribosomal Database Project (RDP-II): Sequences and tools for high-throughput rRNA analysis. *Nucleic Acids Research* **33**: D294–D296.

Compeau GC, & Bartha R. (1985). Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediment. *Applied and Environmental Microbiology* **50**(2): 498–502.

Conway PL. (1995). Microbial ecology of the human large intestine. In: GR Gibson and GT Macfarlanc (eds.). Human Colonic Bacteria: Role in Nutrition, Physiology and Pathology, pp. 1–18. CRC Press, Boca Raton, FL.

Costabile A, Kolida S, Klinder A, Gietl E, Bäuerlein M, Frohberg C, Landschütze V, & Gibson GR. (2010). A double-blind, placebo-controlled, crossover study to establish the bifidogenic effect of a very-long chain inulin extracted from globe artichoke (Cynara scolymus) in healthy human subjects. *British Journal of Nutrition* **104**(7): 1007–1017.

Crittenden R, Laitila A, Forssell P, Mättö J, Saarela M, Mattila-Sandholm T, & Myllärinen, P. (2001). Adhesion of Bifidobacteria to granular starch and its implication in probiotic technologies. *Applied and Environmental Microbiology* **67**(8): 3469–3475.

Cummings JH, & Macfarlane GT. (1991). The control and consequences of bacterial fermentation in the human colon. *Journal of Applied Bacteriology* **70**(6): 443–459.

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, …. & Turnbaugh PJ. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**(7484): 559–563.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, & Lionetti P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Science USA* **107**(33): 14691–14696.

Dethlefsen L, & Relman DA. (2010). Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proceedings of the National Academy of Science USA* **108** (Suppl. 1): 4554–4561.

Dimitrov DV. (2011). The human gutome: Nutrigenomics of the host-microbiome interactions. *OMICS* **15**: 419–430.

Dominguez-Bello, MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, & Knight R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Science USA* **107**(26): 11971–11975.

Dongowski G, Lorenz A, & Proll J. (2002). The degree of methylation influences the degradation of pectin in the intestinal tract of rats and in vitro. *The Journal of Nutrition* **132**(7): 1935–1944.

Doré J, & Blottière H. (2015). The influence of diet on the gut microbiota and its consequences for health. *Current Opinion in Biotechnology* **32**: 195–199.

Dridi B, Fardeau M-L, Ollivier B, Raoult D, & Drancourt M. (2012). *Methanomassiliicoccus luminyensis* gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. *International Journal of Systematic and Evolutionary Microbiology* **62**(8): 1902–1907.

Driscoll CT, Mason RP, Chan HM, Jacob DJ, & Pirrone N. (2013). Mercury as a global pollutant: Sources, pathways, and effects. *Environmental Science & Technology* **47**(10): 4967–4983.

Duncan SH, Hold GL, Barcenilla A, Stewart CS, & Flint HJ. (2002). Roseburia intestinalis sp. nov., a novel saccharolytic, butyrate-producing bacterium from human faeces. *International Journal of Systematic and Evolutionary Microbiology* **52**(5): 1615–1620.

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, ... & Relman DA. (2005). Diversity of the human intestinal microbial flora. *Science* **308**(5728): 1635–1638.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**(19): 2460–2461.

Edwards T, & McBride GC. (1975). Biosynthesis and degradation of methylmercury in human faeces. *Nature* **253**(5491):462–464.

Eggesbø, M. Moen B, Peddada S, Baird D, Rugtveit J, Midtvedt T, Bushel PR, Sekelja M, & Rudi K. (2011). Development of gut microbiota in infants not exposed to medical interventions. *APMIS* **119**(1): 17–35.

EPA (2001). Water quality Criterion for the protection of human health: Methyl-mercury. Environmental Protection Agency (EPA). January 2001. EPA 823-R-10-001, Office of the Science and Technology, Office of Water, Washington, DC.

Erickson PR, & Lin VS. (2015). Research highlights: elucidation of biogeochemical factors influencing methylmercury production. *Environmental Science: Processes & Impacts* **17**(10): 1708–1711.

Eswaran S, Muir J, & Chey WD. (2013). Fiber and functional gastrointestinal disorders. *The American Journal of Gastroenterology* **108**(5): 718–727.

FAO (2010). Food and Agriculture Organization (FAO) of the United Nations. The State of World Fisheries and Aquaculture - 2010; FAO Statistics and Information Service of the Fisheries and Aquaculture Department, Rome, Italy.

FAS (2011). Fishery and Aquaculture Statistics (FAS), 2011; p 415. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fässler C, Arrigoni E, Venema K, Brouns F, & Amadò E. (2006). In vitro fermentability of differently digested resistant starch preparations. *Molecular Nutrition & Food Research* **50**(12): 1220–1228.

Flint HJ, Duncan SH, Scott KP, & Louis P. (2007). Interactions and competition within the microbial community of the human colon: Links between diet and health. *Environmental Microbiology* **9**(5): 1101–1111.

Flint HJ, Scott KP, Louis P, & Duncan SH. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology and Hepatology* **9**(10): 577–589.

Fooks LJ, Fuller R, & Gibson GR. (1999). Prebiotics, probiotics and human gut microbiology. *International Dairy Journal* **9**(1): 53–61.

Fuller R, & Gibson GR. (1997). Modification of the intestinal microflora using probiotics and prebiotics. *Scandinavian Journal of Gastroenterology. Supplement* **222**: 28–31.

Ganther HE. (1978). Modification of methyl mercury toxicity and metabolism by selenium and vitamin E: Possible mechanisms. *Environmental Health Perspectives* **25**: 71–76.

Gibson GR, Ottaway PB, & Rastall RA. (2000). Prebiotics: New Developments in Functional Foods. Chandos Publishing Ltd., Oxford, UK.

Gilmour CC, Elias DA, Kucken AM, Brown SD, Palumbo AV, Schadt CW, & Wall JD. (2011). Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation. *Applied and Environmental Microbiology* **77**(12): 3938–3951.

Gilmour CC, Podar M, Bullock AL, Graham AM, Brown SD, Somenahally AC, ... & Elias DA. (2013). Mercury methylation by novel microorganisms from new environments. *Environmental Science & Technology* **47**(20): 11810–11820.

Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, & Nelson KE. (2006). Metagenomic analysis of the human distal gut microbiome. *Science* **312**(5778): 1355–1359.

Goldin BR. (2011). Probiotics and health: From history to future. In: W Kneifel and S Salminen (eds.). Probiotics and Health Claims, pp. 1–16. Wiley-Blackwell, Chichester, UK.

Graf D, Di Cagno R, Fåk F, Flint HJ, Nyman M, Saarela M, & Watzl B. (2015). Contribution of diet to the composition of the human gut microbiota. *Microbial Ecology in Health and Disease* **26**: 26164 (11 pp).

Graff RD, Falconer MM, Brown DL, & Reuhl KR. (1997). Altered sensitivity of post translationally modified microtubules to methylmercury in differentiating embryonal carcinoma-derived neurons. *Toxicology and Applied Pharmacology* **144**(2): 215–224.

Greenwood RH, Morrill JL, Titgemeyer EC, & Kennedy GA. (1997). A new method of measuring diet abrasion and its effect on the development of the forestomach. *Journal of Dairy Science* **80**(10): 2534–2541.

Grootaert C, Van den Abbeele P, Marzorati M, Broekaert WF, Courtin CM, Delcour JA, … & Van de Wiele T. (2009). Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem. *FEMS Microbiology Ecology* **69**(2): 231–242.

Grootaert C, Marzorati M, Van den Abbeele P, Van de Wiele T, & Possemiers S. (2011). Prebiotics to manage the microbial control of energy homeostasis. *Benef Microbes* **2**(4): 305–318.

Guarner F. (2015). The gut microbiome: What do we know?. *Clinical Liver Disease* **5**(4): 86–90.

Guerra A, Etienne-Mesmin L, Livrelli V, Denis S, Blanquet-Diot S, & Alric M. (2012). Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends in Biotechnology* **30**(11): 591–600.

Ha E, Basu N, Bose-O'Reilly S, Dórea JG, McSorley E, Sakamoto M, & Chan HM. (2017). Current progress on understanding the impact of mercury on human health. *Environmental Research* **152**: 419–433.

Hamaker BR, & Tuncil YE. (2014). A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *Journal of Molecular Biology* **426**(23): 3838–3850.

Harada M. (1978). Congenital Minamata disease: intrauterine methylmercury poisoning. *Teratology* **18**(2): 285–288.

Hentges DJ. (1992) Gut flora and disease resistance. In R Fuller (ed.). Probiotics, The Scientific Basis, pp. 87–110. Chapman and Hall, London, UK.

Hildebrandt MA, Hoffmann C, Sherrill–Mix SA, Keilbaugh SA, Hamady M, Chen YY, ... & Wu GD. (2009). High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* **137**(5): 1716–1724.

Hillman B. (2004). Role of Gut Bacteria in Human Toxicology and Pharmacology. CRC Press, Boca Raton, FL.

Hintelmann H, Evans RD, & Villeneuve JY. (1995). Measurement of mercury methylation in sediments by using enriched stable mercury isotopes combined with methylmercury determination by gas chromatography - inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry* **10**(9): 619–624.

Hintelmann H, & Evans RD. (1997). Application of stable isotopes in environmental tracer Studies - Measurement of monomethylmercury (CH_3Hgþ) by isotope dilution ICP-MS and detection of species transformation. *Fresenius*' *Journal of Analytical Chemistry* **358**(3): 378–385.

Hintelmann H, & Ogrinc N. (2003). Determination of stable mercury isotopes by ICP-MS and their application in environmental studies. In: Y Cai and OC Braids (eds.). Biogeochemistry of Environmentally Important Trace Elements. *ACS Symposium Series* **835**: Ch 21, pp. 321–338. American Chemical Society, Washington, DC.

Himeno S, Watanabe C, Hongo T, Suzuki T, Naganuma A, & Imura N. (1989). Body size and organ accumulation of mercury and selenium in young harbor seals (Phoca vitulina). *Bulletin of Environmental Contamination and Toxicology* **42**(4): 503–509.

Hooper LV, Littman DR, & Macpherson AJ. (2012). Interactions between the microbiota and the immune system. *Science* **336**(6086): 1268–1273.

Hughes SA, Shewry PR, Li L, Gibson GR, Sanz ML, & Rastall RA. (2007). *In vitro* fermentation by human fecal microflora of wheat arabinoxylans. *Journal of Agricultural and Food Chemistry* **55**(11): 4589–4595.

Hughes SA, Shewry PR, Li L, Gibson GR, McCleary BV, & Rastall RA. (2008). *In vitro* fermentation of oat and barley derived betaglucans by human faecal microbiota. *FEMS Microbiology Ecology* **64**(3): 482–493.

Hwang CY. (2006). *Flectobacillus lacus sp. nov.*, isolated from a highly eutrophic pond in Korea. *International Journal of Systematic and Evolutionary Microbiology* **56**(6):1197–1201.

Imura N, & Naganuma A. (1985). Mode of modifying action of selenite on toxicity and behavior of mercury and other metals. *Nutrition Research* (Suppl. 1): 499–507.

InSug O, Datar S, Koch CJ, Shapiro IM, & Shenker BJ. (1997). Mercury compounds inhibit human monocytes function by inducing apoptosis: Evidence for formation of reactive oxygen species, development of mitochondrial membrane permeability transition and loss of reductive reserve. *Toxicology* **124**(3): 211–224.

Jensen S, & Jernelöv A. (1969). Biological methylation of mercury in aquatic organisms. *Nature* **223**(5207): 753–754.

Jernberg C, Löfmark S, Edlund C, & Jansson JK. (2010). Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology* **156**(11): 3216–3223.

Jugo S, Maljković T, & Kostial K. (1975). Influence of chelating agents on the gastrointestinal absorption of lead. *Toxicology and Applied Pharmacology* **34**(2): 259–263.

Jugo S. (1977). Metabolism of toxic heavy metals in growing organisms: A review. *Environmental Research* **13**(1): 36–46.

Kajiwara Y, Yasutake A, Adachi T, & Hirayama K. (1996). Methylmercury transport across the placenta via neutral amino acid carrier. *Archives in Toxicology* **70**(5): 310–314.

Karlsson CLJ, Molin G, Cilio CM, & Ahrné S. (2011). The pioneer gut microbiota in human neonates vaginally born at term-A pilot study. *Pediatric Research* **70**(3): 282–286.

Kello D, & Kostial K. (1973). The effect of milk diet on lead metabolism in rats. *Environmental Research* **6**(3): 355–360.

Kerrin EJ, Gilmour CC, Roden E, Suzuki MT, Coates JD, & Mason RP. (2006). Mercury methylation by dissimilatory iron-reducing bacteria. *Applied Environmental Microbiology* **72**(12): 7919–7921.

Khan MT, Duncan SH, Stams AJ, van Dijl JM, Flint HJ, & Harmsen HJ. (2012). The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic-anoxic interphases. *ISME Journal* **6**(8): 1578–1585.

Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, & Ley RE. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Science USA* **108**(Suppl 1): 4578–4585.

Laird BD, Shade C, Gantner N, Chan HM, & Siciliano SD. (2009). Bioaccessibility of mercury from traditional northern country foods measured using an in vitro gastrointestinal model is independent of mercury concentration. *Science of the Total Environment* **407**(23): 6003–6008.

Landry TD, Doherty RA, & Gates AH. (1979). Effects of three diets on mercury excretion after methylmercury administration. *Bulletin of Environmental Contamination and Toxicology* **22**(1): 151–158.

Lapina VA, Sheshko PM, & Dontsov AE. (2000). Phytosorbent prepared from sunflower seed husks prevents mercuric chloride accumulation in kidney and muscle of adult rabbits. *Archives of Environmental Health* **55**(1): 48–50.

Lebet V, Arrigoni E, & Amado R. (1998). Measurement of fermentation products and substrate disappearance during incubation of dietary fibre sources with human faecal flora. *Lebensmittel-Weissenschaft Und-Technologie-Food Science and Technology* **31**: 473–479.

Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, & Mazmanian SK. (2013). Bacterial colonization factors control specificity and stability of the gut microbiota. *Nature* **501**(7467): 426–429.

Levander OA, & Cheng L. (1980). Micronutrient interactions, vitamins, minerals, and hazardous elements. *New York Academy of Sciences* **355**: 1–372.

Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, & Gordon JI. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences USA* **102**(31): 11070–11075.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, & Gordon JI. (2008). Evolution of mammals and their gut microbes. *Science* **320**(5883): 1647–1651.

Licht TR, Hansen M, Bergström A, Poulsen M, Krath BN, Markowski J, ... & Wilcks A. (2010). Effects of apples and specific apple components on the cecal environment of conventional rats: Role of apple pectin. *BMC Microbiology* **10**(1): 13 (11 pp).

Liebert CA, Wireman J, Smith T, & Summers AO. (1997). Phylogeny of mercury resistance (*mer*) operons of gram-negative bacteria isolated from the fecal flora of primates. *Applied and Environmental Microbiology* **63**(3): 1066–1076.

Lin C-C, Yee N, & Barkay T. (2012). Microbial transformations in the mercury cycle. In: C Liu, C Yong and N O'driscoll (eds.). Environmental Chemistry and Toxicology of Mercury, pp. 155–191. John Wiley & Sons, Inc., Hoboken, NJ.

Liszt K, Zwielehner J, Handschur M, Hippe B, Thaler R, & Haslberger AG. (2009). Characterization of bacteria, clostridia and Bacteroides in faeces of vegetarians using qPCR and PCR-DGGE fingerprinting. *Annals of Nutrition and Metabolism* **54**(4): 253–257.

Lopez-Legarrea P, Fuller NR, Zulet MA, Martinez JA, & Caterson ID. (2014). The influence of Mediterranean, carbohydrate and high protein diets on gut microbiota composition in the treatment of obesity and associated inflammatory state. *Asia Pacific Journal of Clinical Nutrition* **23**(3): 360–368.

Louis P, Scott KP, Duncan SH, & Flint HJ. (2007). Understanding the effects of diet on bacterial metabolism in the large intestine. *Journal of Applied Microbiology* **102**(5): 1197–1208.

Lozupone C, & Knight R. (2005). UniFrac: A new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology* **71**(12): 8228–8235.

Macfarlane GT, & Gibson GR. (1994). Metabolic activities of normal colonic flora. In: SAW Gibson (ed.). Human Health: The Contribution of Microorganisms, pp. 17–52. Springer Verlag, London, UK.

Macfarlane S, & Macfarlane GT. (2004). Bacterial diversity in the human gut. *Advances in Applied Microbiology* **54**: 261–289.

Macfarlane GT, & Macfarlane S. (2007). Models for intestinal fermentation: Association between food components, delivery systems, bioavailability and functional interactions in the gut. *Current Opinion in Biotechnology* **18**(2): 156–162.

Macfarlane GT, & Macfarlane S. (2011). Fermentation in the human large intestine: Its physiologic consequences and the potential contribution of prebiotics. *Journal of Clinical Gastroenterology* **45**(S1): 120–127.

Maier BR, Flynn MA, Burton GC, Tsutakawa RK, & Hentges DJ. (1974). Effects of a high-beef diet on bowel flora: A preliminary report. *American Journal of Clinical Nutrition* **27**(12): 1470–1474.

Marathe N, Shetty S, Lanjekar V, Ranade D, & Shouche Y. (2012). Changes in human gut flora with age: An Indian familial study. *BMC Microbiology* **12**(1): 222 (10 pp).

Martínez I, Kim J, Duffy PR, Schlegel VL, & Walter J. (2010). Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS ONE* **5**(11): e15046 (11 pp).

Matijašić BB, Obermajer T, Lipoglavšek L, Grabnar I, Avguštin G, & Rogelj I. (2014). Association of dietary type with fecal microbiota in vegetarians and omnivores in Slovenia. *European Journal of Nutrition* **53**(4): 1051–1064.

McBurney MI, & Thompson LU. (1987). Effect of human faecal inoculum on in vitro fermentation variables. *British Journal of Nutrition* **58**(2): 233–243.

McBurney MI, & Thompson LU. (1989). Effect of human fecal donor on in vitro fermentation variables. *Scandinavian Journal of Gastroenterology* **24**(3): 359–367.

McMurdie PJ, & Holmes S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**(4): e61217 (11 pp).

Mergler D, Anderson HA, Chan LHM, Mahaffey KR, Murray M, Sakamoto M, & Stern AH. (2007). Methylmercury exposure and health effects in humans: A worldwide concern. *AMBIO: Journal of the Human Environment* **36**(1): 3–11.

Miettinen JK, Rahola T, Hattula T, Rissanen K, & Tillander M. (1971). Elimination of ^{203}Hg-methylmercury in man. *Annals of Clinical Research* **3**(2): 116–122.

Miura K, & Imura N. (1989). Mechanism of cytotoxicity of methylmercury: With special reference to microtubule disruption. *Biological Trace Element Research* **21**(1): 313–316.

Modler HW. (1994). Bifidogenic factors - sources, metabolism and applications. *International Dairy Journal* **4**(5): 383–407.

Molly K, Vande Woestyne M, & Verstraete W. (1993). Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Applied Microbiology and Biotechnology* **39**(2): 254–258.

Molly K, Vande Woestyne M, De Smet I, & Verstraete W. (1994). Validation of the simulator of the human intestinal microbial ecosystem (SHIME) reactor using microorganism-associated activities. *Microbial Ecology in Health and Disease* **7**(4): 191–200.

Montgomery L. (1988). Isolation of human colonic fibrolytic bacteria. *Letters in Applied Microbiology* **6**(3): 55–57.

Muegge BD, Kuczynski J, Knights D, Clemente JC, González A, Fontana L, Henrissat B, Knight R, & Gordon JI. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* **332**(6032): 970–974.

Murphy EF, Cotter PD, Hogan A, O'Sullivan O, Joyce A, Fouhy F, Clarke SF, Marques TM, O'Toole PW, Stanton C, Quigley EM, Daly C, Ross PR, O'Doherty RM, & Shanahan F. (2013). Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. *Gut* **62**(2): 220–226.

Nakamura I, Hosokawa K, Tamra H, & Miura T. (1977). Reduced mercury excretion with feces in germfree mice after oral administration of methyl mercury chloride. *Bulletin of Environmental Contamination and Toxicology* **17**(5): 528–533.

Norseth T, & Clarkson TW. (1970). Biotransformation of methylmercury salts in the rat studied by specific determination of inorganic mercury. *Biochemical Pharmacology* **19**(10): 2775–2783.

NRC (2000): National Research Council (NRC). Toxicological Effects of Methylmercury. National Academy Press, Washington, DC. Doi: http://www.nap.edu/openbook.php?isbn=0309071402 (accessed February 15, 2017).

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, ... & Wagner H. (2016). Vegan: Community Ecology Package. R package version 2.3–4.

Ostlund K. (1969). Studies on the metabolism of methylmercury in mice. *Acta Pharmacologica et Toxicologica* **27**(Suppl. 1): 1–132.

Ou J, Carbonero F, Zoetendal EG, DeLany JP, Wang M, Newton K, Gaskins HR, & O'Keefe SJ. (2013). Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *American Journal of Clinical Nutrition* **98**(1): 111–120.

Parks JM, Johs A, Podar M, Bridou R, Hurt RA, Smith SD, Tomanicek SJ, Qian Y, Brown SD, Brandt CC, Palumbo AV, Smith JC, Wall JD, Elias DA, & Liang L. (2013). The genetic basis for bacterial mercury methylation. *Science* **339**(6125): 1332–1335.

Passos CJ, Mergler D, Fillion M, Lemire M, Mertens F, Guimaraes JRD, & Philibert A. (2007). Epidemiologic confirmation that fruit consumption influences mercury exposure in riparian communities in the Brazilian Amazon. *Environmental Research* **105**(2): 183–193.

Peraza MA, Ayala-Fierro F, Barber DS, Casares E, & Rael LT. (1998). Effects of micronutrients on metal toxicity. *Environmental Health Perspectives* **106**(Suppl. 1): 203–216.

Poulain AJ, & Barkay T. (2013). Cracking the mercury methylation code. *Science* **339**(6125): 1280–1281.

Price MN, Dehal PS, & Arkin AP. (2010). Fast Tree 2 – approximately maximum-likelihood trees for large alignments. *PLoS ONE* **5**(3): e9490 (10 pp).

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, ... & Glöckner FO. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**(D1): D590–D596.

Queipo-Ortuño MI, Boto-Ordóñez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, Estruch R, ... & Tinahones FJ. (2012). Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *American Journal of Clinical Nutrition* **95**(6): 1323–1334.

Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Grietje H, & Petra L. (2009). Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *British Journal of Nutrition* **101**(4): 541–550.

Rao MV, Chinoy NJ, Suthar MB, & Rajvanshi MI. (2001). Role of ascorbic acid on mercuric chroride-induced genotoxicity in human blood cultures. *Toxicology In Vitro* **15**(6): 649–654.

Rao MV, & Sharma PSN. (2001). Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice. *Reproductive Toxicology* **15**(6): 705–712.

R Core Team (2015). RA Language and Environment Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Doi: http://www.cran.r-project.org.

Rice DC. (1989). Delayed neurotoxicity in monkeys exposed developmentally to methylmercury. *Neurotoxicology* **10**(4): 645–650.

Rice DC. (1989). Brain and tissue levels of mercury after chronic methyl mercury exposure in the monkey. *Journal of Toxicology and Environmental Health* **27**(2): 189–198.

Rideout JR, He Y, Navas-Molina JA, Walters WA, Ursell LK, Gibbons SM, Chase J, McDonald D, ... & Caporaso JG. (2014). Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. *PeerJ* **2**: e545 (25 pp).

Roberfroid MB. (1998). Prebiotics and synbiotics: Concepts and nutritional properties. *British Journal of Nutrition* **80**(4): 197–202.

Robert C, & Bernalier-Donadille A. (2003). The cellulolytic microflora of the human colon: evidence of microcrystalline cellulose-degrading bacteria in methane excreting subjects. *FEMS Microbial Ecology* **46**(1): 81–89.

Rose DJ, Keshavarzian A, Patterson JA, Venkatachalam M, Gillevet P, & Hamaker, BR. (2009). Starch-entrapped microspheres extend in vitro fecal fermentation, increase butyrate production, and influence microbiota pattern. *Molecular Nutrition & Food Research* **53**(S1): S121–S130.

Rothenberg SE, Korrick SA, & Fayad R. (2015). The influence of obesity on blood mercury levels for U.S. non-pregnant adults and children: NHANES 2007–2010. *Environmental Research* **138**: 173–180.

Rothenberg SE, Keiser S, Ajami NJ, Wong MC, Gesell J, Petrosino JF, & Johs A. (2016). The role of gut microbiota in fetal methylmercury exposure: Insights from a pilot study. *Journal of Toxicology Letters* **242**: 60–67.

Rowland M, Benet LZ, & Graham GG. (1973). Clearance concepts in pharmacokinetics. *Journal of Pharmacokinetics and Biopharmaceutics* **1**(2): 123–136.

Rowland IR, Grasso P, & Davies MJ. (1975). The methylation of mercuric chloride by human intestinal bacteria. *Experientia* **31**(9): 1064–1065.

Rowland IR, Robinson RD, Doherty RA, & Landry TD. (1983). Are developmental changes in methylmercury metabolism and excretion mediated by the intestinal microflora? In: TW Clarkson, GF Nordberg and PR Sagar (eds.). Reproductive and Developmental Toxicity of Metals. pp. 745–758. Springer, New York, NY.

Rowland IR, Robinson RD, & Doherty RA. (1984). Effects of diet on mercury metabolism and excretion in mice given methylmercury: Role of gut flora. *Archives of Environmental Health* **39**(6): 401–408.

Rowland IR. (1986). Reduction by the gut microflora of animals and man. *Biochemical Pharmacology* **35**(1): 27–32.

Russell WR, Gratz SW, Duncan SH, Holtrop G, Ince J, Scobbie L, Duncan G, …& Flint HJ. (2011). High-protein, reduced-carbohydrate weight loss diets promote metabolite profiles likely to be detrimental to colonic health. *American Journal of Clinical Nutrition* **93**(5): 1062–1072.

Sajilata MG, Singhal RS, & Kulkarni PR. (2006). Resistant starch – A review. *Comprehensive Reviews in Food Science and Food Safety* **5**(1): 1–17.

Sakamoto M, Kubota M, Liu XJ, Murata K, Nakai K, & Satoh H. (2004). Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. *Environmental Science and Technology* **38**(14): 3860–3863.

Sakamoto M, Murata K, Tsuruta K, Miyamoto K, & Akagi H. (2010). Retrospective study on temporal and regional variations of methylmercury concentrations in preserved umbilical cords collected from inhabitants of the Minamata area, Japan. *Ecotoxicology and Environmental Safety* **73**(6): 1144–1149.

Sanchez JI, Marzorati M, Grootaert C, Baran M, Van Craeyveld V, Courtin CM, ... & Van de Wiele T. (2009). Arabinoxylan-oligosaccharides (AXOS) affect the protein/carbohydrate fermentation balance and microbial population dynamics of the Simulator of Human Intestinal Microbial Ecosystem. *Microbial Biotechnology* **2**(1): 101–113.

Sarafian TA, Bredesen DE, & Verity MA. (1996). Cellular resistance to methylmercury. *Neurotoxicology* **17**(1): 27–36.

Schaedler RW. (1973). The relationship between the host and its intestinal microflora. *Proceedings of the Nutrition Society* **32**(2): 41–47.

Schaefer JK, Kronberg RM, Morel FMM, & Skyllberg U. (2014). Detection of a key Hg methylation gene, *hgcA*, in wetland soils. *Environmental Microbiol*ogy *Reports* **6**(5): 441–447.

Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, Zwahlen MC, Desiere F, Bork P, Delley M, Pridmore RD, & Arigoni F. (2002). The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proceedings of the National Academy of Science USA* **99**(22): 14422–14427.

Schnorr SL, Candela M, Rampelli S, Centanni M, Consolandi C, Basaglia G, Turroni S, Biagi E, … & Crittenden AN. (2014). Gut microbiome of the Hadza hunter-gatherers. *Nature Communications* **5**: 3654 (12 pp).

Schwiertz A, Lehmann U, Jacobasch G, & Blaut M. (2002). Influence of resistant starch on the SCFA production and cell counts of butyrate-producing *Eubacterium* spp. in the human intestine. *Journal of Applied Microbiology* **93**(1): 157–162.

Scott KP, Martin JC, Campbell G, Mayer C-D, & Flint HJ. (2006). Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium '*Roseburia inulinivorans*'. *Journal of Bacteriology* **188**(12): 4340–4349.

Seko Y, Miura T, Takahashi T., & Koyama T. (1981). Methyl mercury decomposition in mice treated with antibiotics. *Acta Pharmacologica et Toxicologica* **49**(4): 259–265.

Selin NE. (2009). Global biogeochemical cycling of mercury: A review. *Annual Review of Environment and Resources* **34**(1): 1–457.

Shanker G, & Aschner M. (2001). Identification and characterization of uptake systems for cystine and cysteine in cultured astrocytes and neurons: Evidence for methylmercury-targeted disruption of astrocyte transport. *Journal of Neuroscience Research* **66**(5): 998–1002.

Shannon CE. (1948). A mathematical theory of communication. *Bell System Technical Journal* **27**: 379–423, and 623–656.

Shinohara K, Ohashi Y, Kawasumi K, Terada A, & Fujisawa T. (2010). Effect of apple intake on fecal microbiota and metabolites in humans. *Anaerobe* **16**(5): 510–515.

Simpson EH. (1949). Measurement of diversity. *Nature* **163**(4148): 688–688.

Smith SD, Bridou R, Johs A, Parks JM, Elias DA, Hurt Jr. RA, Brown SD, Podar M, & Wall JD. (2015). Site-directed mutagenesis of HgcA and HgcB reveals amino acid residues important for mercury methylation. *Applied Environmental Microbiology* **81**(9): 3205–3217.

Sommer F, & Bäckhed F. (2013). The gut microbiota - Masters of host development and physiology. *Nature Reviews Microbiology* **11**(4): 227–238.

Sone N, Larsstuvold MK, & Kagawa Y. (1977). Effect of methyl mercury on phosphorylation, transport, and oxidation in mammalian mitochondria. *Journal of Biochemistry (Tokyo)* **82**(3): 859–868.

Sonnenburg JL, Angenent LT, & Gordon JI. (2004). Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? *Nature Immunology* **5**(6): 569–573.

Steer T, Carpenter H, Tuohy K, & Gibson GR. (2000). Perspectives on the role of the human gut microbiota and its modulation by pro- and prebiotics. *Nutrition Research Reviews* **13**(2): 229–254.

Stern AH, & Smith AE. (2003). An assessment of the cord blood: maternal blood methylmercury ratio: implications for risk assessment. *Environmental Health Perspectives* **111**(12): 1465–1470.

Stewart ML, & Slavin JL. (2006). Molecular weight of guar gum affects short-chain fatty acid profile in model intestinal fermentation. *Molecular Nutrition and Food Research* **50**(10): 971–976.

Suda I, & Hirayama K. (1992). Degradation of methyl- and ethylmercury into inorganic mercury by hydroxyl radical produced from rat liver microsomes. *Archives of Toxicology* **66**(6): 398–402.

Suda I, & Takahashi H. (1986). Enhanced and inhibited biotransformation of methyl mercury in the rat spleen. *Toxicology and Applied Pharmacology* **82**(1): 45–52.

Suzuki T, Yonemoto J, Satoh H, Naganuma A, Imura N, & Kigama T. (1984). Normal organic and norganic mercury levels in the human feto-placental system. *Journal of Applied Toxicol*ogy **4**(5): 249–252.

Swidsinski A, Loening-Baucke V, Verstraelen H, Osowska S, & Doerffel Y. (2008). Biostructure of fecal microbiota in healthy subjects and patients with chronic idiopathic diarrhea. *Gastroenterology* **135**(2): 568–579.

Tan H, & O'Toole PW. (2015). Impact of diet on the human intestinal microbiota. *Current Opinion in Food Science* **2**: 71–77.

Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JP, ... & Leclerc M. (2009). Towards the human intestinal microbiota phylogenetic core. *Environmental Microbiology* **11**(10): 2574–2584.

Tremaroli V, & Bäckhed F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature* **489**(7415): 242–249.

Tuohy K. (2000). Measurement of DNA transfer in the gut using in vitro and in vivo models. PhD Thesis, University of Surrey, Guildford, UK.

Tuohy KM, Finlay RK, Wynne AG, & Gibson GR. (2001). A human volunteer study on the prebiotic effects of HP-inulin—faecal bacteria enumerated using fluorescent *in situ* hybridisation (FISH). *Anaerobe* **7**(3): 113–118.

Tuohy KM, Kolida S, Lustenberger AM, & Gibson GR. (2001). The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides - A human volunteer study. *British Journal of Nutrition* **86**(3): 341–348.

Urano T, Naganuma A, & Imura N. (1988). Species differences in biliary excretion of methylmercury - Role of non-protein sulfhydryls in bile. *Research Communications in Chemical Pathology and Pharmacology* **62**(2): 339–351.

Usuki F, Yasutake A, Umehara F, Tokunaga H, Matsumoto M, Eto K, Ishiura S, & Higuchi I. (2001). In vivo protection of a water soluble derivative of vitamin E, Trolox, against methyl mercuryintoxication in the rat. *Neuroscience Letters* **304**(3): 199–203.

Vahter M, Akesson A, Lind B, Bjors U, Schutz A, & Berglund M. (2000). Longitudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. *Environmental Research* **84**(2): 186–194.

Van den Abbeele P, Gérard P, Rabot S, Bruneau A, El Aidy S, Derrien M, ... & Van de Wiele T. (2011). Arabinoxylans and inulin differentially modulate the mucosal and luminal gut microbiota and mucin-degradation in humanized rats. *Environmental Microbiology* **13**(10): 2667–2680.

Vendrame S, Guglielmetti S, Riso P, Arioli S, Klimis-Zacas D, & Porrini M. (2011). Six-week consumption of a wild blueberry powder drink increases bifidobacteria in the human gut. *Journal of Agricultural and Food Chemistry* **59**(24): 12815–12820.

Vonk JW, & Sijpesteijn AK. (1973). Studies on the methylation of mercuric chloride by pure cultures of bacteria and fungi. *Antonie van Leeuwenhoek* **39**(1): 505–513.

Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, Brown D, Stares MD, … & Flint HJ. (2011). Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME Journal* **5**(2): 220–230.

Walsh CT, Distefano MD, Moore MJ, Shewchuk LM, & Verdine GL. (1988). Molecular basis of bacterial resistance to organomercurial and inorganic salts. *FASEB Journal* **2**(2): 124–130.

Wang Y, Boyd E, Crane S, Lu-Irving P, Krabbenhoft D, King S, ... & Barkay T. (2011). Environmental conditions constrain the distribution and diversity of archaeal merA in Yellowstone National Park, Wyoming, USA. *Microbial Ecology* **62**(4): 739–752.

Watanabe S, & Koessel S. (1993). Colon cancer: An approach from molecular epidemiology. *Journal of Epidemiology* **3**(2): 47–61.

Wedekind KJ, Mansfield HR, & Montgomery L. (1988). Enumeration and isolation of cellulolytic and hemicellulolytic bacteria from human feces. *Applied and Environmental Microbiology* **54**(6): 1530–1535.

Whanger PD. (1992). Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. *Journal of Trace Elements and Electrolytes in Health and Disease* **6**(4): 209–221.

Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, … & Lewis JD. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**(6052): 105–108.

Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, Hooper LV, & Gordon JI. (2003). A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. *Science* **299**(5615): 2074–2076.

Xu Z, & Knight R. (2014). Dietary effects on human gut microbiome diversity. *British Journal of Nutrition* **133**(Suppl 0): S1–S5.

Yamamoto R, Suzuki T, Satoh H, & Kawai K. (1986). Generation and dose as modifying factors of inorganic mercury accumulation in brain, liver, and kidneys of rats fed methylmercury. *Environmental Research* **41**(1): 309–318.

Yee S, & Choi BH. (1996). Oxidative stress in neurotoxic effects of methylmercury poisoning. *Neurotoxicology* **17**(1): 17–26.

Yorifuji T, Tsuda T, Inoue S, Takao S, & Harada M. (2011). Long-term exposure to methylmercury and psychiatric symptoms in residents of Minamata, Japan. *Environment International* **37**(5): 907–913.

Yoshino Y, Mozai T, & Nakao K. (1966). Biochemical changes in the brain in rats poisoned with an alkymercury compound, with special reference to the inhibition of protein synthesis in brain cortex slices. *Journal of Neurochemistry* **13**(11): 1223–1230.

Zhang YHP, & Lynd LR. (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnology and Bioengineering* **88**(7): 797–824.

Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, Mao Y, Zhang X, Pang X, Wei C, Zhao G, Chen Y, & Zhao L. (2010). Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME Journal* **4**(2): 232–241.

Zhao L. (2013). The gut microbiota and obesity: From correlation to causality. *Nature Reviews Microbiology* **11**(9): 639–647.

Ziemer CJ, & Gibson GR. (1998). Overview of probiotics, prebiotics and synbiotics in the functional food concept. *International Dairy Journal* **8**(5-6): 473–479.

Zimmer J, Lange B, Frick JS, Sauer H, Zimmermann K, Schwiertz A, ... & Enck, P. (2012). A vegan or vegetarian diet substantially alters the human colonic faecal microbiota. *European Journal of Clinical Nutrition* **66**(1): 53–60.

Zoetendal EG, Raes J, van den Bogert B, Arumugam M, Booijink CCGM, Troost FJ, Bork P, Wels M, de Vos WM, & Kleerebezem M. (2012). The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *ISME Journal* **6**(7): 1415–1426.

Appendix A

Supplementary data 1:

Qiime pipeline for 16s rRNA data processing

```
#!/bin/bash
#$ -N Ria_June_2016
#$ -l mem_free=200G
#$ -q abaqus.q
#$ -j y
#$ -S /bin/bash

#1 check the mapping file
validate_mapping_file.py -m ~/matti/Ria_June_2016/Ria_June_2016_map.txt -o
~/matti/Ria_June_2016/mapping_output

#2 split libraries according to the mapping file
split_libraries.py -m ~/matti/Ria_June_2016/mapping_output/Ria_June_2016_map_corrected.txt
-f ~/matti/Ria_June_2016/Ria_June_2016.fasta -q ~/matti/Ria_June_2016/Ria_June_2016.qual -
o ~/matti/Ria_June_2016/split_library_output -b 8 -l 300 -L 600

#3 vsearch chimera check against reference SILVA123 core alignment, output both non-chimeric
(forwarded into pipeline) and chimeric sequences
vsearch --uchime_ref ~/matti/Ria_June_2016/split_library_output/seqs.fna --db
~/matti/SILVA123_QIIME_release/core_alignment/core_alignment_SILVA123_gold.fasta --
nonchimeras ~/matti/Ria_June_2016/seqs_chimeras_filtered.fna --
chimeras  ~/matti/Ria_June_2016/seqs_chimeric.fna

#4 open reference OTU picking using default QIIME settings, uclust at 97% identity and
SILVA123 97% clustered database, ran in parallel where possible
pick_open_reference_otus.py -i ~/matti/Ria_June_2016/seqs_chimeras_filtered.fna -r
~/matti/SILVA123_QIIME_release/rep_set/rep_set_16S_only/97/97_otus_16S.fasta -o
~/matti/Ria_June_2016/openref_picking_output/ --prefilter_percent_id 0.6 -a

#5 (optional) a quick summary of the OTU table for QC
biom summarize-table -i
~/matti/Ria_June_2016/openref_picking_output/otu_table_mc2_w_tax_no_pynast_failures.biom
-o ~/matti/Ria_June_2016/openref_picking_output/otu_table_summary.txt

#6 remove singleton OTUs
filter_otus_from_otu_table.py -i
~/matti/Ria_June_2016/openref_picking_output/otu_table_mc2_w_tax_no_pynast_failures.biom
-o ~/matti/Ria_June_2016/openref_picking_output/otu_table_no_singletons.biom -n 2
```

#7 remove OTUS that matched to chloroplasts and mitochondria from the OTU table (biom table ready!)
filter_taxa_from_otu_table.py -i
~/matti/Ria_June_2016/openref_picking_output/otu_table_no_singletons.biom -o
~/matti/Ria_June_2016/openref_picking_output/otu_table_noclo.biom -n
D_2__Chloroplast,D_4__Mitochondria

#8 (for tree file creation) remove the sequences of these otus from the pynast aligned .fasta file
filter_fasta.py -f
~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs/rep_set_aligned_pfiltered.fasta -b ~/matti/Ria_June_2016/openref_picking_output/otu_table_noclo.biom -o
~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs/rep_set_aligned_pfiltered_noclo.fasta

#9 (for tree file creation) (this command originates from primer prospector 1.0.1) clean up the pynast aligned .fasta file (remove the aligning)
clean_fasta.py -f
~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs/rep_set_aligned_pfiltered_noclo.fasta -o ~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs

#10 (for tree file creation) realign the rep set .fasta file without singletons, the chloroplast and mitochondria to the SILVA123 core alignment
align_seqs.py -i
~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs/rep_set_aligned_pfiltered_noclo_filtered.fasta -o
~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs/realigned_root -t
~/matti/SILVA123_QIIME_release/core_alignment/core_alignment_SILVA123.fasta

#11 (for tree file creation) filter gap characters (necessary for generating an useful tree!)
filter_alignment.py -i
~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs/realigned_root/rep_set_aligned_pfiltered_noclo_filtered_aligned.fasta -o
~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs/realigned_root/ -e 0.10 -g 0.80

#12 (for tree file creation) make a tree of the phylogeny
make_phylogeny.py -i
~/matti/Ria_June_2016/openref_picking_output/pynast_aligned_seqs/realigned_root/rep_set_aligned_pfiltered_noclo_filtered_aligned_pfiltered.fasta -o
~/matti/Ria_June_2016/openref_picking_output/rep_phylo_root.tre

#13 add metadata to the OTU table (and move it to a new folder "PHYLOSEQ" for exporting)
cp ~/matti/Ria_June_2016/openref_picking_output/otu_table_noclo.biom
~/matti/PHYLOSEQ/Ria_June_2016.biom

#14 convert the OTU table to a format readable by phyloseq

biom convert -i ~/matti/PHYLOSEQ/Ria_June_2016.biom -o
~/matti/PHYLOSEQ/Ria_June_2016_json.biom --to-json

#15 copy the newly made tree to the "PHYLOSEQ" folder
cp ~/matti/Ria_June_2016/openref_picking_output/rep_phylo_root.tre
~/matti/PHYLOSEQ/Ria_June_2016_rep_phylo_root.tre

#16 (optional) a quick summary of the OTU table for QC
biom summarize-table -i ~/matti/PHYLOSEQ/Ria_June_2016.biom -o
~/matti/PHYLOSEQ/Ria_June_2016_summary.txt

#17 (optional) convert the OTU table to tab limited tsv
biom convert -i ~/matti/PHYLOSEQ/Ria_June_2016_json.biom -o
~/matti/PHYLOSEQ/Ria_June_2016_tsv.txt" --to-tsv --header-key taxonomy

---

Supplementary Table S1: Comparison of microbial diversity estimation of the 16s rRNA gene libraries at 3% dissimilarity from Illumina sequencing analysis.

Group	Reads	OTUs	Shannon Index	Simpson Index
T0 balanced	48217	2511	4.853	0.974
T0 fat	60419	2925	4.893	0.975
T0 carbohydrate	57916	2823	4.863	0.975
T0 protein	88422	3348	4.805	0.972
T0 Hg balanced	68761	2904	4.516	0.958
T0 Hg fat	84222	3142	4.558	0.960
T0 Hg carbohydrate	83524	3230	4.546	0.957
T0 Hg protein	81343	3292	4.553	0.958
T96 balanced	119033	1664	1.881	0.439
T96 fat	82739	1282	1.675	0.383
T96 carbohydrate	105434	1714	2.039	0.475
T96 protein	112110	1617	1.782	0.411
T96 Hg balanced	106377	1699	1.869	0.434
T96 Hg fat	83808	1432	1.767	0.405
T96 Hg carbohydrate	126067	1594	1.611	0.373
T96 Hg protein	106207	1746	2.032	0.499

Supplementary Figure S1

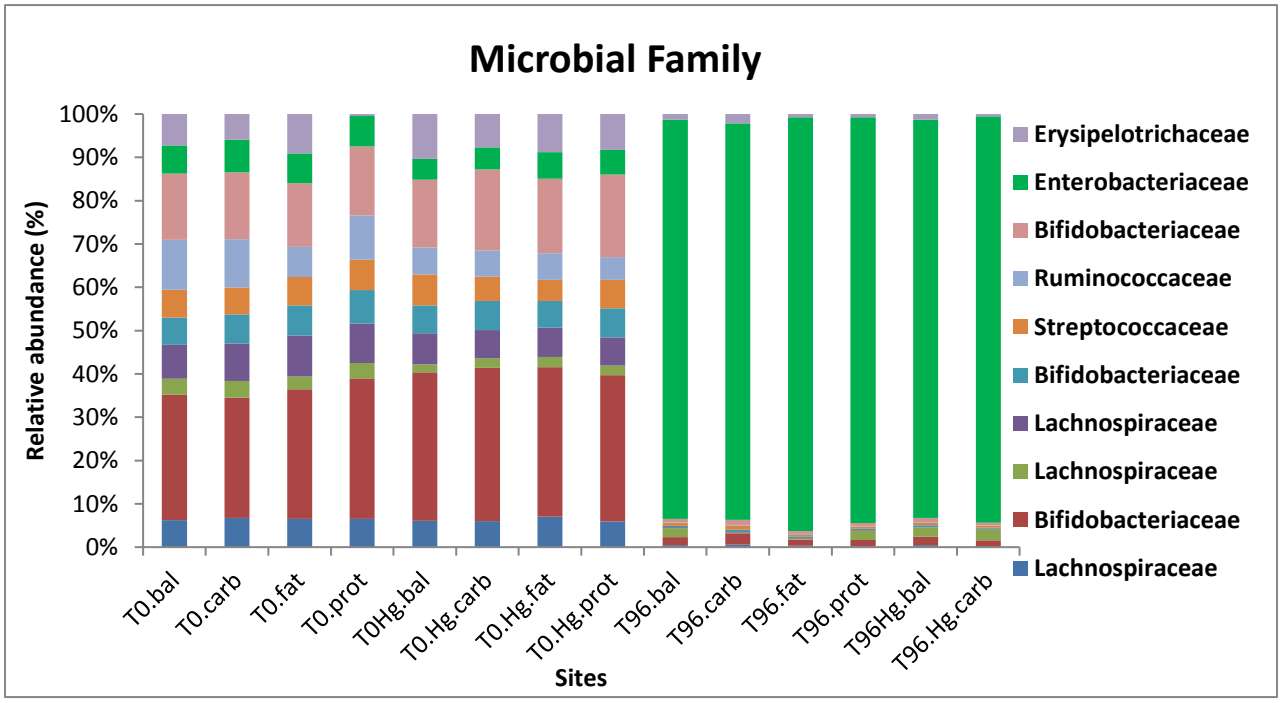

Figure S1: The relative abundance of major (top 10) taxonomic groups at family levels across all treatments - the two most dominant groups belongs to Bifidobacteriaceae accounting for 30% at time 0 h, and Enterobacteriaceae accounting for 90% at time 96 h.