An Ecological Approach to Understanding Gut Microbiota and Macrobiota Interactions

Emily Louise Pascoe

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"Truth is born into this world only with pangs and tribulations, and every fresh truth is received unwillingly."

Alfred R. Wallace

Thesis Summary

Microbiota (community of micro-organisms) and macrobiota (parasitic helminths) are ubiquitous in the gut, and both elicit a number of positive and negative affects on host health. Despite a plethora of studies investigating microbiota and macrobiota, research rarely considers how these two sympatric communities interact. Given that microbiota and macrobiota affect host health, and are both under increasing evolutionary pressures that may affect how the two interact e.g., antibiotic and anthelmintic treatments, it is timely to investigate microbiota-macrobiota interactions. This thesis uses an ecological approach to understand microbiota-macrobiota interactions in a wild system. First, a review of animal gut microbiota literature established the current research landscape of this topic, which highlighted the lack of research on wild animals, despite the advantages it can provide, e.g., as model systems (Chapter 2). In addition, field experiments used perturbation in a wild rodent to tease apart microbiota-macrobiota interactions. The affect of helminth removal (using anthelmintic) on microbiota was investigated, with diversity and composition of bacterial communities remaining stable, with the exception of faecal microbiota, following anthelmintic treatment (Chapter 3). Following perturbation of the microbiota (using antibiotic), helminth abundance decreased, but prevalence, fecundity and size all increased (Chapter 4). Helminths were found to be associated with a microbiota that exhibits intra- and inter-specific variation in diversity and composition, driven by gut location, but composition of helminth microbiota also significantly differed to that of the gut (Chapter 5). Finally, the effect of faecal microbiota on helminth development was tested; egg hatching was less successful and slower in self faeces, compared to non-self faeces from another individual, indicating that faeces have some resistance to helminth development (Chapter 6). This thesis highlights the importance of considering the interactions of the two main components of the gut

biome when manipulating either microbiota or macrobiota, for experimental or treatment purposes.

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Chapter 1

General introduction: Gut microbiota and macrobiota

"One touch of nature makes the whole world kin."

William Shakespeare

1.1 Chapter overview

In this introductory chapter, an overview of the literature on gut microbiota and macrobiota is provided, before giving a brief synopsis of the current knowledge on microbiota-macrobiota interactions, which are given more attention in each of the relevant data chapters. The premise for using a wild rodent study system, the yellow-necked mouse (*Apodemus flavicollis*) is discussed. Finally, the overarching aims of the thesis are presented, which collectively intend to further the knowledge on microbiota-macrobiota interactions using an ecological approach.

1.2 Gut microbiota acquisition and functions

Every multicellular organism is colonised by a community of micro-organisms, which may include bacteria, single celled eukaryotes, fungi and viruses (Marchesi and Ravel, 2015). Collectively, these micro-organisms are often inaccurately described as the 'microbiome', however this more specifically describes the cumulative genome of these micro-organisms and the environment with which they interact, and instead 'metataxome' or 'microbiota' more accurately describes the taxonomic composition of a microbial community (Marchesi and Ravel, 2015). Microbes inhabit many internal and external niches including the skin (Grice *et al.*, 2009), oral cavities (Dewhirst *et al.*, 2010) and pulmonary system (Barfod *et al.*, 2013) of animals, and likewise the roots (Kristin and Miranda, 2013), seeds (Johnston-Monje and Raizada, 2011) and the entire above-ground phyllosphere of plants (Lindow and Brandl, 2003). The microbial communities inhabiting these different niches have a highly specific composition, and microbiota variation has even been observed between each tooth of an individual (Bik *et al.*, 2010). The number of these microbial cells often exceeds the number of host cells; for example, in mammals microbes are estimated to outnumber host cells by around ten times (Palmer *et al.*, 2007).

The microbiota of the gut is the most dense and diverse to colonise vertebrates. Humans typically possess 10^{11}-10^{12} microbes/ml of luminal content (Palmer *et al.*, 2007), which is comprised of an estimated 500 to 1,000 species (Hrncir *et al.*, 2008), and equates to a genome that consists of 150 times more genes than that of a human (Gill *et al.*, 2006). Gut microbes are continually acquired throughout the lifespan of an individual. Although it was previously believed that the gut was sterile until birth (Dominguez-Bello *et al.*, 2010; Koenig *et al.*, 2011), it is now accepted that some intrauterine vertical transmission of gut bacteria is likely to occur (Jiménez *et al.*, 2008). However, the first critical inoculum with significant impacts on the host is received during birth (Dominguez-Bello *et al.*, 2010; Jakobsson *et al.*, 2014). Indeed, in humans the mode of birth can have lifelong consequences; the gut of vaginally delivered babies are initially colonised by maternal faecal and vaginal microbes (Dominguez-Bello *et al.*, 2010; Jakobsson *et al.*, 2014). However, individuals delivered by caesarean section are more susceptible to autoimmune diseases, which is thought to be a consequence of the fact that their gut is instead first colonised by microbes typically found on the skin, which affects the maturation of the immune system (Dominguez-Bello *et al.*, 2010; Jakobsson *et al.*, 2014; Figure 1.1).

Due to changes in diet, development of the immune system and high levels of environmental transmission associated with the first years of life, the gut microbiota of humans is highly dynamic until about three years of age, after which time the microbiota remains relatively stable but can still fluctuate (Koenig *et al.*, 2011). Factors that influence the microbiota include host characteristics, e.g., age (Biagi *et al.*, 2013), gender (Mueller *et al.*, 2006; Markle *et al.*, 2013) and genetics (Khachatryan *et al.*, 2008) or environmental characteristics such as diet (Gibso*n et a*l., 2004), and seasonality (Carey *et al.*, 2013; Jia *et al.*, 2013). As a result of experience and exposure, the microbiota can therefore vary greatly between individuals within the same species,

and within an individual throughout time. Consequently, despite an effort to categorise the gut microbiota into 'enterotypes', based on statistical clustering patterns of microbial taxa (Arumugam *et al.*, 2011), this approach is controversial. Critics claim that microbiota cannot easily be categorised into groups, as variation between individuals exists along a gradient (Jeffery *et al.*, 2012).

Microbiota studies have been propelled by, but also have driven, advances in microbiology technologies that can characterise microbiota composition and functions, such as the development of multi-'omic platforms, including metataxonomics and metagenomics, resulting in a snowball effect on microbiota research and knowledge (Marchesi and Ravel, 2015). The subsequent plethora of gut microbiota studies are largely driven by the knowledge that this bacterial community is vital for host health and physiological processes, and research on this 'microbial organ' (Bäckhed *et al.*, 2005) has rapidly expanded, and continues to do so. The relationship between the host and its microbiota is largely mutualistic: in return for nutrients from gut contents, the microbiota is involved in chemical functions within the body, including the digestion of complex carbohydrates, production of secondary metabolites such as vitamins, and the regulation of sex hormones (Schluter and Foster, 2012; Markle *et al.*, 2013; Figure 1.1). However, gut microbiota may also exert negative impacts on the host, particularly if there is an imbalance in microbial composition (termed 'dysbiosis'; Figure 1.1). Dysbiosis has been implicated in a number of non-infectious diseases such as Crohn's disease (Dicksved *et al.*, 2008), obesity (Ley *et al.*, 2005), and both type 1 and type 2 diabetes (Qin *et al.*, 2012; Peng *et al.*, 2014). In addition, not all microbes in the gut are beneficial to the host, and pathogenic micro-organisms sometimes present in the gut include some strains of *Escherichia coli*, while other micro-organisms, such as *Clostridium difficile*, may in usual circumstances be benign, but become pathogenic under certain

dysbiotic conditions, such as when the numbers of bacteria which normally suppress its over-growth are low, allowing *C. difficile* to proliferate (Aas *et al.*, 2003).

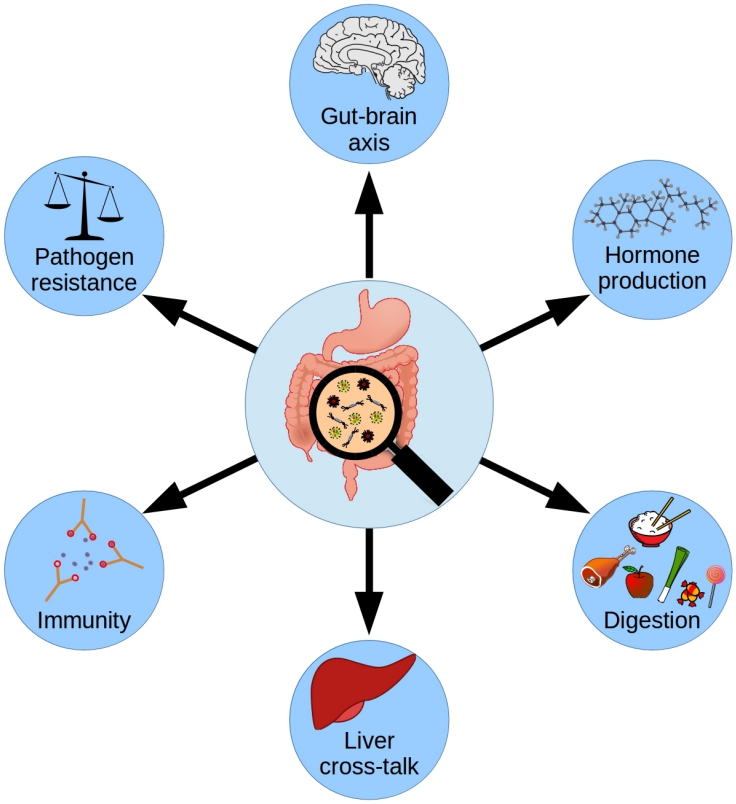

Figure 1.1: Gut microbiota studies have been driven by the knowledge that it is involved in many crucial functions within the host, including immunity, digestion and hormone production, as well as interactions with organs such as the brain and liver. However, microbiota composition can become dysbiotic, leading to pathogenesis and disease.

Microbiota can also play a role in cognition, emotion and behaviour exhibited by the host (Figure 1.1). The gut-brain axis is a widely accepted concept that describes the bidirectional interactions that occur between the microbiota and the central nervous system, which occur due to a complex network of cytokines, hormones and the neural system (reviewed by Bercik *et al.*, 2012). This gut-brain intercommunication can result in behavioural phenotypes in the host associated with microbiota composition; for example, when newly hatched Kudzu bugs (*Megacopta cribraria*) are

prevented from ingesting maternal symbiotic capsules, they exhibit wandering behaviour in search of the probiotic (Hosokawa *et al.*, 2008). Moreover, in the laboratory, a reduction in behaviours associated with anxiety have been observed in both germ-free mice (Diaz Heijtz *et al.*, 2011), and in mice administered the probiotic *Lactobacillus rhamnosus* (Bravo *et al.*, 2011). Non-infectious diseases that affect cognition and behaviour have been linked to certain microbiota profiles; e.g., autism has been associated with higher abundances and diversity of *Clostridium* spp. in faeces (Finegold *et al.*, 2002), and infection with specific enteric pathogens has been associated with decreased cognitive abilities (Gareau *et al.*, 2011). In addition, the microbiota has a strong association with another vital organ; the liver, for example through a cross-talk of bile acids, lipopolysaccharides and deoxycholic acids, high levels of which may be reached during gut dysbiosis and can lead to damage and disease of the liver (reviewed by Bourzac, 2014).

Of particular importance for host health are the interactions between the gut microbiota and the immune system. The only physical barrier separating potential pathogens ingested by the host from other organs is a 30 μm single layer of intestinal epithelial cells (Cahenzli *et al.*, 2012). The microbiota must be continuously monitored by immune cells to maintain homoeostasis and prevent dysbiosis and pathogenic infection. This very microbiota is also vital for the development of these immune cells and the immune system: gut microbes promote lymphocyte and immunoglobulin production (Round and Mazmanian, 2009; Cahenzli *et a*l., 2012), influence the ability of the gut to act as a physical barrier against pathogens (Deplancke and Gaskins, 2001), are involved in the development of immune structures such as Peyer's patches (Kamada and Núñez, 2013), and can influence the ability of other bacteria to colonise the gut (Rolfe *et al.*, 1981). Indeed many studies using animal models have found that gnotobiotic mammals (i.e., those with a

sterile gut, or which possess a limited and specific microbiota) are unable to develop a fully functioning immune system (Schluter and Foster, 2012).

1.3 Manipulation of the gut microbiota

Given the impact of gut microbiota on host health as described above (e.g., Round and Mazmanian, 2009; Bercik *et al.*, 2012; Schluter and Foster, 2012; Markle *et al.*, 2013) a great deal of research has been dedicated to understanding how it can be manipulated or modulated to incite health benefits and treat disease. Antibiotics have been administered to kill or prevent the proliferation of pathogenic bacteria since their discovery in the early 1900s (Aminov, 2010; Hauser, 2012). However antibiotics often function on a 'broad-spectrum', and inadvertently induce changes in the entire microbial composition, by affecting non-target and non-pathogenic bacteria. Changes in microbiota associated with antibiotic administration can exacerbate and even cause dysbiosis by affecting symbiotic bacteria, e.g., by affecting microbes involved in immunity (Francino, 2016).

The impacts on microbiota resulting from antibiotic treatment can be long-term; in humans antibiotic associated perturbation of gut microbiota has been reported up to four years after antibiotic administration (Kilkkinen *et al.*, 2002; Jakobsson *et al.*, 2010). Furthermore, bacterial resistance to antibiotics is increasing at a rate greater than drug development (Shlaes, 2010), and concerns associated with overuse of antibiotics (McEwen and Fedorka-Cray, 2002; Dibner and Richards, 2005) led to a ban in 2006 within the EU on their use as a feed-additive to promote growth in livestock (Anadón, 2006). However, antibiotics continue to be used in alarming quantities; hundreds of tonnes are used annually in salmon farms in Chile alone (Cabello *et al.*, 2013). In addition, little if anything is currently known about the potential wider implications of

antibiotic use, for example the effect on the whole gut biome composition (including viruses, protozoa and macroparasites). Treatments which promote the natural community of the microbiota, such as probiotics and prebiotics, are being tested and employed to treat dysbiosis, and can also incite other benefits such as improved immunity and growth (Edens, 2003; Patterson and Burkholder, 2003; Geraylou *et al.*, 2013).

Probiotics (viable micro-organisms derived from maternal symbiotic capsules, faeces, or from culture) are taken by humans and animals alike to directly improve gut microbiota composition, and probiotics have been consumed by humans for centuries in fermented foods such as dairy products (Parvez *et al.*, 2006) and preserved meats, albeit without specific intention. As knowledge on beneficial microbes has grown, testing and subsequent production of various isolated bacteria species as probiotics, particularly lactic acid bacteria (Naidu *et al.*, 1999), has become an area of interest for food and pharmaceutical companies (Saxelin, 2008). Probiotics containing *Lactobacillus, Bifidobacterium* and *Enterococcus* are frequently administered to livestock due to their health inducing benefits; anaerobic gut bacteria lead to weight gain and improved food conversion efficiency (Fuller, 1989). Consumption of probiotics can also be observed in wildlife, such as the Kudzu bug (*Megacopta cribraria*) and bumble bees (*Bombus terrestris*), which may consume probiotics to prevent disease and improve general gut health (Hosokawa *et al.*, 2008; Koch and Schmid-Hempel, 2011).

Although probiotics are typically composed of a single species or strain of bacteria, it is possible to administer an entire community of micro-organisms by faecal microbiota transplant (FMT). FMT involves transplanting faeces from a healthy individual into the gut of a recipient suffering dysbiosis, whereby bacteria in the faeces act like a multi-species probiotic to supplement the

microbiota of the recipient. FMT has proved successful in relieving symptoms of otherwise difficult to treat infections such as *C. difficile* (Aas *et al.*, 2003; MacConnachie *et al.*, 2009). Despite many years of anecdotal and small-scale study claims of FMT success (e.g., Eiseman *et al.*, 1958) there are concerns regarding the safety of FMT, due to the lack of studies on long-term impacts and potential risks associated with transferring a whole microbiota to an individual. For example, infectious pathogens from the faecal donor may also be transferred to the recipient. Evidence also suggests that microbiota may revert to its previous composition if FMT is not regularly administered (Aas *et al.*, 2003; Rawls *et al.*, 2006; Brandt and Aroniadis, 2013).

'Bacterial interference' is another branch of probiotics, which exploit the antagonistic interactions between bacterial species known to 'interfere' with a pathogen. Bacterial interference is mainly based on the concept that in order to infect a host, bacteria must adhere to a biological surface (Reid and Sobel, 1987); for example, a healthy urinary bladder is sterile because bacteria are unable to attach to the internal walls due to micturition (constant voiding of urine through bladder from kidneys; Reid and Sobel, 1987). Certain bacterial species (administered as a probiotic) can prevent colonisation of pathogenic bacteria by 'interfering' with the adhesion of the pathogen to the host gut (Reid *et al.*, 2001). Interference may be achieved by bacteria out-competing the pathogen for host-cell-binding sites and nutrients, inhibiting the toxin-receptor interactions of the pathogen or simply by killing it (Reid *et al.*, 2001). A similar concept to bacterial interference is 'paratransgenesis', whereby symbionts of a host are genetically modified to express effector molecules against a pathogen (Coutinho-Abreu *et al.*, 2010). As such, paratransgenesis may have a role in biocontrol for disease vectors, as the host is less competent at vectoring pathogens as a consequence. Paratransgenesis has the added advantage that transmission throughout a population

of insect vectors can be self-perpetuating through vertical or coprophagous transmission (Coutinho-Abreu *et al.*, 2010).

Alternatively, the gut microbiota may be modulated indirectly, for example through diet or prebiotics. Prebiotics are substances such as carbohydrates, which are ingested to provide a growth substrate for specific microbes already present in the gut, in order to regain or maintain intestinal homoeostasis (Pourabedin *et al.*, 2014). A prebiotic can be administered in combination with a probiotic (a 'synbiotic'), to amalgamate the benefits of both, often with enhanced results. For example *Bifidobacteria*, beneficial for its saccharolytic (Gibson *et al.*, 1995) and mucosal barrier enhancing properties (Cani *et al.*, 2007) can be administered together with oligofructose, a carbohydrate readily available to stimulate *Bifidobacteria* growth (Collins and Gibson, 1999). Diet can act as an arguably less refined prebiotic, and both diet composition and quantity can have major impacts on microbiota which are both rapid and reproducible (David *et al.*, 2014; Sonnenburg and Bäckhed, 2016). As such, diet can be a powerful tool for modulating microbiota and has been the topic of many microbiota studies (e.g., Desai *et al.*, 2012; Deusch *et al.*, 2014; Roggenbuck *et al.*, 2014).

1.4 Sharing the gut: parasitic helminths – the macrobiota

Macroparasites (multicellular parasites), like micro-organisms, are ubiquitous: billions of humans are infected with helminths worldwide (Hotez *et al.*, 2006). Infections are equally pervasive in animals, with dramatic economic consequences in livestock; for example, in the United States of America the annual loss associated with nematode infection of sheep alone is estimated to be USD 42 million (Waller, 2006). Helminths can infect many organs of the body, such as the liver, brain

and lungs. Of interest here is the macroparasite community that is, spatially, most closely associated with the gut microbiota; the enteric parasitic helminths or the 'macrobiota'.

Although many macroparasitic infections can be relatively benign, helminths can induce a variety of sub-lethal effects on the host such as malnutrition, appetite loss, anaemia and reduced fecundity, and consequently are considered one of the main causes of poor productivity and ill health in domesticated animals (Shetty, 2010; Sutherland and Scott, 2010). Despite these negative effects, it is important to note that an absence or reduction in helminths, as observed in some westernised societies (where there is better access to healthcare and flushing toilets, breaking the life-cycle of faecal-oral transmitted species), may also have negative effects on the host (Bilbo *et al.*, 2011). A rise in auto- and hyperimmune disease prevalence has been associated with reduced contact with helminths (Bilbo *et al.*, 2011), which has been linked to the fact that helminth infection stimulates a cellular immune response in the host (Yazdanbakhsh *et al.*, 2002). The resulting increase in levels of immunoglobulin antibodies is similar to that observed during autoimmunity; however, the physiological response differs: the immune regulatory network is strengthened with a consequential response by T-helper 2 cells to allergens, in effect 'training' the immune system to elicit an appropriate response to pathogens (Yazdanbakhsh *et al.*, 2002).

An individual may be infected by macroparasites from a number of sources. Trophic transmission can occur by drinking or eating contaminated foodstuffs (Udeh, 2004). Other macroparasites such as *Ancylostoma duodenale* require direct physical contact with the host in order to infect it, by penetrating the skin (Bethony *et al.*, 2006). Macroparasites may also be transmitted between host species; zoonotic macroparasites are those that spill-over from animal hosts into humans, and some parasite species infect an intermediate host during development before infecting the

definitive host (Legesse and Erko, 2004; Pullola *et al.*, 2006). Depending on the life-cycle of the parasite, transmission and infection may occur at different life stages. Typically, the life-cycle of parasitic helminths undergoes three separate stages: the egg, at least one larval stage, and the adult stage (Engelkirk *et al.*, 2011), although not all of these life stages are necessarily parasitic and may occur in the environment (Figure 1.2). For example *Trichuris suis* is infective after the egg has hatched in the environment, and is parasitic from the first larval stage (L1); however, *Heligmosomoides polygyrus* hatches in the environment but is not infective until undergoing two larval moults (L3 larval stage; Acton, 2011; Figure 1.2). Meanwhile, some parasites can infect the host during the egg stage, for example *Trichuris trichiura* (Bethony *et al.*, 2006).

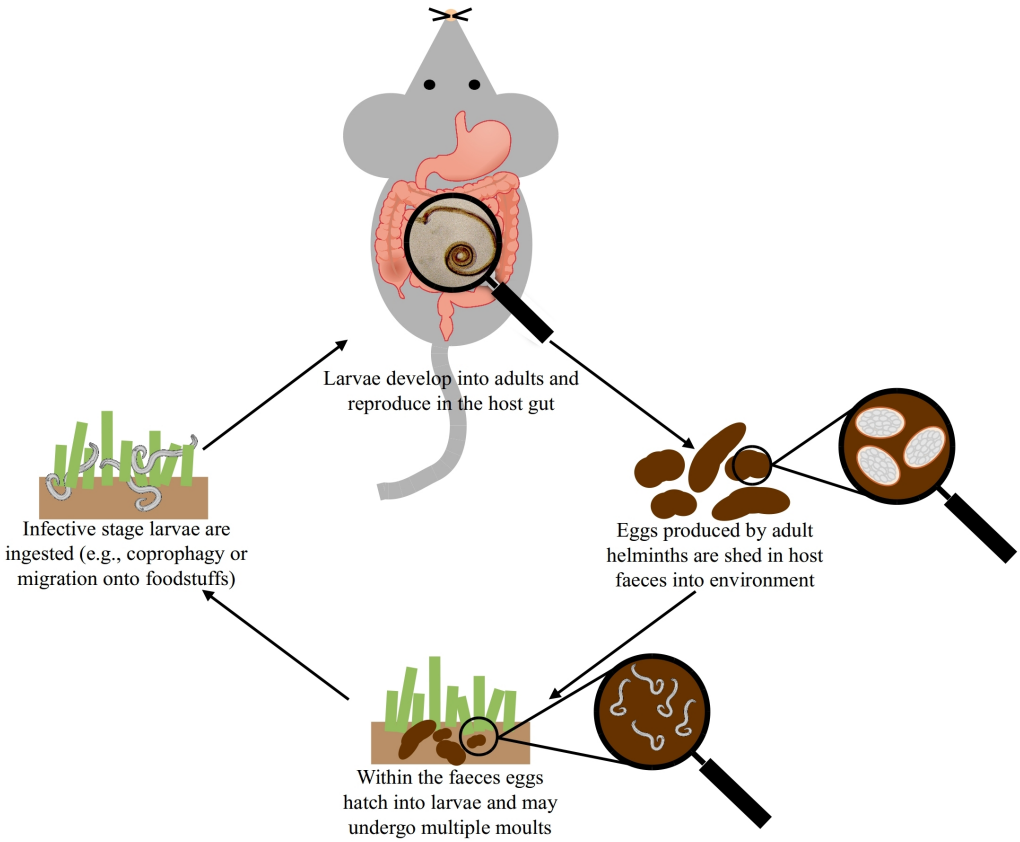

Figure 1.2: An overview of the life-cycle of a typical parasitic helminth that infects the gut. Generally, the life-cycle undergoes three separate stages: the egg, at least one larval stage and the adult stage, although not all of these life stages are necessarily parasitic, and may occur in the environment. For example, *Heligmosomoides polygyrus* eggs are shed in host faeces and hatch in the environment. Following multiple larval stages, the infective larvae are ingested by the host, where they develop and produce eggs in the gut.

1.5 Microbiota-macrobiota interactions – what do we know so far?

The microbiota and macrobiota share the gut in space and time, and have co-evolved. As these two communities have profound positive and negative effects on host health, research on the interactions between the microbiota and macrobiota is starting to grow, but still very little is known. Evidence thus far clearly indicates that the microbiota and parasitic helminths do interact in a number of ways (Glendinning *et al.*, 2014; Reynolds *et al.*, 2015). Microbiota composition can affect the susceptibility of an individual to helminth infection (Martínez-Gómez *et al.*, 2009; Hayes *et al.*, 2010; Coêlho *et al.*, 2013), and in turn infection can influence the microbial community, usually by increasing bacterial diversity (Cebra, 1999; Maizels *et al.*, 2004; Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Rausch *et al.*, 2013; Kreisinger *et al.*, 2015). Although increased bacterial diversity associated with helminth infection has been attributed to microbiota-immunity interplay (Cebra, 1999; Maizels *et al.*, 2004; Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Rausch *et al.*, 2013), helminths may in addition act as a vector of pathogenic bacteria into the gut (Perkins and Fenton, 2006; Lacharme-Lora *et al.*, 2009a, 2009b). Helminths may spend at least one life stage in the environment and acquire their own microbiota (Walk *et al.*, 2010; Figure 1.2), which could be transmitted to the gut of the host (Perkins and Fenton, 2006; Lacharme-Lora *et al.*, 2009a, 2009b). However, in order to successfully infect a host in the first instance, the helminth may require bacteria to complete their life-cycle, for example to hatch (Hayes *et al.*, 2010; Koyama, 2013; Vejzagić *et al.*, 2015), or to develop to the adult stage of the parasite (Weinstein *et al.*, 1969). Consequently, some helminths are unable to form persistent infections in germ-free mice (gnotobiotic; sterile or have a reduced and specific gut microbial composition; Wescott, 1968; Chang and Wescott, 1972).

Investigating microbiota-macrobiota interactions is particularly timely given that these two communities are under increasing evolutionary pressures (e.g., imposed by antibiotic and anthelmintic treatment) with unknown consequences for the rest of the gut biome. In addition, recent advances in technologies have greatly facilitated the study of microbiota (Marchesi and Ravel, 2015). Much of the research on microbiota-macrobiota interactions summarised above has been performed on laboratory animals. This is due to the practical and ethical restrictions associated with experimentation and research using humans (McGuire *et al.*, 2008), an approach which is often necessary to tease apart interactions within a system, as illustrated by traditional ecological experiments (Paine, 1966). Given that many variables such as environmental and host characteristics affect microbiota (Gibson *et al.*, 2004; Khachatryan *et al.*, 2008; Jakobsson *et al.*, 2010; Carey *et al.*, 2013; Markle *et al.*, 2013) and macrobiota composition (Bundy and Golden, 1987; Bundy *et al.*, 1988; Schalk and Forbes, 1997), carefully controlled studies are vital. On the other hand, laboratory studies are limited as they lack context in the complex environment of the 'real world' (Amato, 2013). This thesis aims to investigate the interactions that occur between natural microbiota and macrobiota by using a wild animal model system which has both communities intact.

1.6 Investigating microbiota-macrobiota interactions in a free-living system

The yellow-necked mouse (*Apodemus flavicollis*) was used in this thesis as a wild model system to investigate microbiota-macrobiota interactions. *Apodemus flavicollis* is normally associated with mature deciduous woodland habitat (Ferrari *et al.*, 2004). Fieldwork to collect samples for the data chapters was performed within multiple grids/transects at four field sites in; San Michele all'Adige (46°11'24.8"N, 11°08'27.6"E; 46°11'31.6"N 11°08'20.2"E and 46°11'17.9"N 11°08'16.2"E), Cavedine (45°59'10.6"N, 10°57'47.1"E; 45°58'30.8"N, 10°57'22.0"E and

45°59'21.2"N, 10°57'59.6"E) Pietramurata (46°00'52.2"N, 10°55'27.7"E; 46°00'47.7"N, 10°55'40.7"E and 46°01'01.4"N, 10°55'22.8"E) and Lagolo on Monte Bondone (46°03'28.6"N, 11°00'47.9"E), all in the Province of Trento, situated in the Region of Trentino-Alto Adige, of the northeastern Italian Alps. The parasitic helminth community of the *A. flavicollis* gut has been well described previously, and studies on natural gut microbiota composition have also been performed on this species, including in the chosen study area (Ferrari, 2005; Perkins *et al.*, 2008; Ferrari *et al.*, 2009; Kreisinger *et al.*, 2015).

1.7 Thesis aims

This thesis uses an ecological approach to understand the interactions between gut microbiota and gut macrobiota (Figure 1.3). The thesis is composed of five self-contained data chapters; one literature review (Chapter 2) and four experimental chapters, all of which are in preparation for publication (Chapter 3-6; Figure 1.3). First, a literature review was performed on non-human animal gut microbiota which established the research landscape of animal microbiota studies. The experimental chapters follow, which largely used manipulation as a means to tease apart microbiota-macrobiota interactions. Firstly, a field experiment was perfromed to examine the effect of helminth perturbation (by anthelmintic) on microbiota composition is presented (Chapter 3), and this is followed by a field study on the effect of microbiota depletion (by antibiotic) on helminth prevalence, burden and fecundity (Chapter 4, Figure 1.3). Next, the diversity and composition of helminth-associated microbiota was investigated, and compared to that of the host gut (Chapter 5). The effect of faecal microbiota on helminth development was then explored, whereby probability and rate of helminth egg development in microbiota of ‘self’ faeces of the host, and of ‘non-self’ faeces from another individual were compared (Chapter 6, Figure 1.3). Finally, the cumulative results of these data are discussed in context, and any subsequent research

questions and implications are addressed (Chapter 7). In Appendix A.8 additional work is presented, which although not directly part of this thesis, has contributed to it. With the exceptions of Chapters 1 and 7, each chapter has been written as a manuscript in preparation for submission, and Chapter 2 is currently in press for publication in ISME Journal. Therefore, this has led to some overlap in content between chapters, particularly with respect to methods (Figure 1.3).

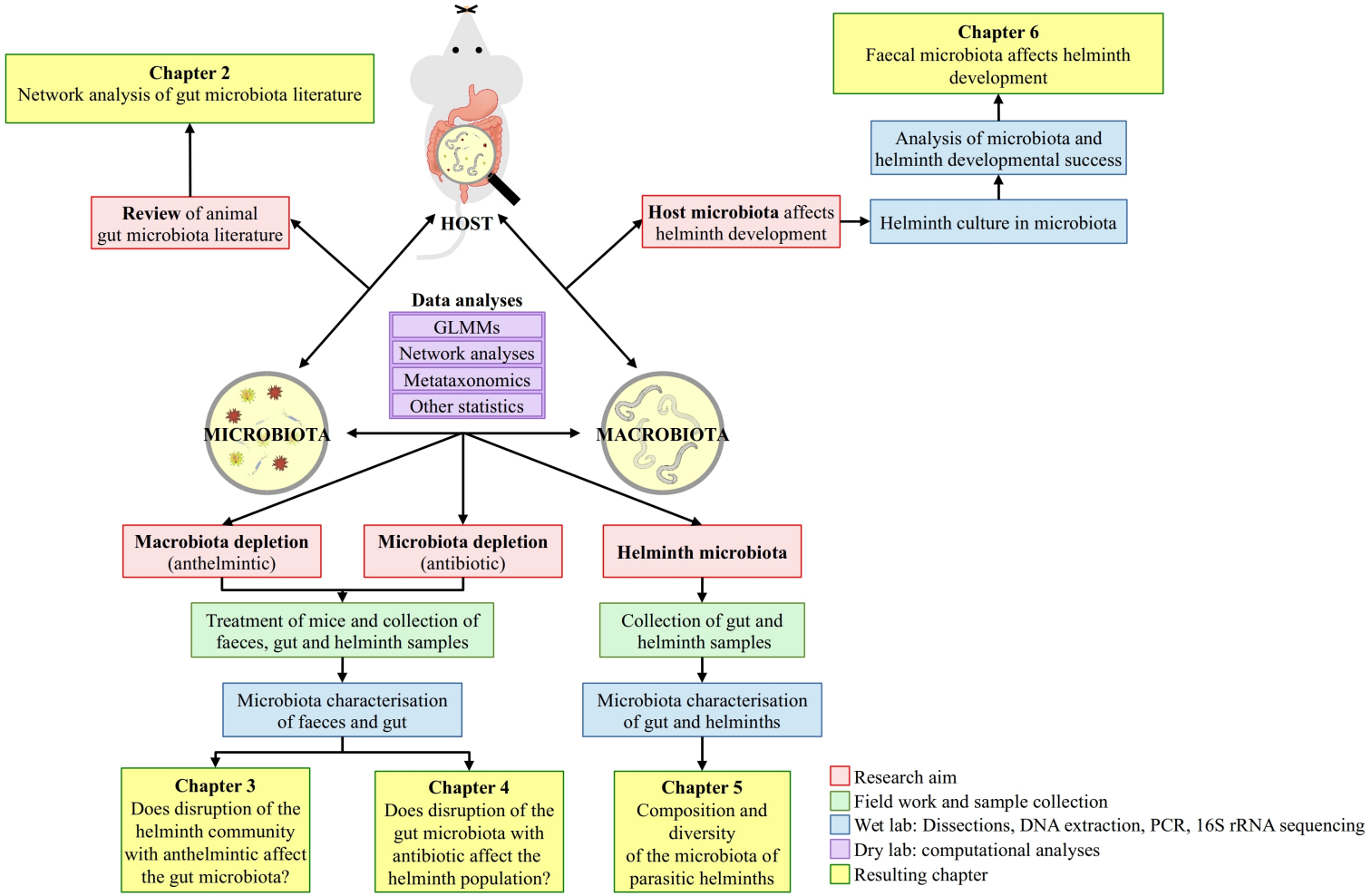

Figure 1.3: Overview of aims of data chapters to understand microbiota-macrobiota interactions, and how samples will be collected and analysed to satisfy each question posed. The thesis is composed of one literature review and four experimental chapters, of which three involve experimental manipulation of the microbiota and/or macrobiota, and one involves observation of the natural helminth and host microbiota. Biometric data is recorded for all individuals, in addition to the collection of helminth and microbiota samples (e.g., for statistical analyses and 16S rRNA sequencing). Data are analysed using a series of statistical methods, including generalised linear mixed model.

Chapter 2

Network analysis of gut microbiota literature

"In all works on Natural History, we constantly find details of the marvellous adaptation of animals to their food, their habits, and the localities in which they are found."

Alfred R. Wallace

2.1 Abstract

A wealth of human studies have demonstrated the importance of gut microbiota to health. Research on non-human animal gut microbiota is now increasing, but what insight does it provide? We reviewed 650 publications from this burgeoning field (2009-2016) and determined that animals driving this research were predominantly 'domestic' (48.2%), followed by 'model' (37.5%), with least studies on 'wild' (14.3%) animals. Domestic studies largely experimentally perturbed microbiota (81.8%) and studied mammals (47.9%), often to improve animal productivity. Perturbation was also frequently applied to model animals (87.7%), mainly mammals (88.1%), for forward translation of outcomes to human health. In contrast, wild animals largely characterised natural, unperturbed microbiota (79.6%), particularly in pest or pathogen vectoring insects (42.5%). We used network analyses to compare the research foci of each animal group. 'Diet' was the main focus in all three, but to different ends: to enhance animal production (domestic), to study non-infectious diseases (model), or to understand microbiota composition (wild). Network metrics quantified model animal studies as most interdisciplinary, while wild animals incorporated the fewest disciplines. Overall, animal studies, especially model and domestic, cover a broad array of research. Wild animals, however are the least investigated, but offer under-exploited opportunities to study 'real-life' microbiota.

2.2 Review of literature

2.2.1 The dawn of modern microbiota research

Technological advances in multi-'omic platforms such as metataxonomics and metagenomics, have helped fuel the recent expansion of microbiota research (Marchesi and Ravel, 2015), especially on humans, as exemplified by large-scale efforts such as The Human Microbiome

Project, started in 2007 (Peterson *et al.*, 2009). Research on microbiota from non-human habitats has followed: in 2010 the Earth Microbiome Project (www.earthmicrobiome.org) was initiated to document microbial diversity across multiple biomes (Gilbert *et al.*, 2014). Studies focusing on microbiota of the gut have especially captivated scientific interest; it is the most dense and diverse microbial community of the body, is influenced by a range of intrinsic and extrinsic variables including diet, genetics and environmental factors (Khachatryan *et al.*, 2008; Phillips, 2009; Claesson *et al.*, 2012; Bright and Bulgheresi, 2010), and is vital to health and development (Round and Mazmanian, 2009; Lozupone *et al.*, 2012). In recent years non-human animal gut microbiota studies have started to emerge, for example, characterising the microbiota of giant pandas, *Ailuropoda melanoleuca*, to make microbial comparisons across age groups (Tun *et al.*, 2014), or of the European honey bee, *Apis mellifera*, to understand the role of bacteria in nutrition (Engel *et al.*, 2012). But, what other species have been studied, and why? Given this field is burgeoning it is timely to take stock of the non-human animal gut microbiota literature and determine the research landscape thus far.

Here, we ask 'what drives research in animal gut microbiota?' by quantifying the subject as a domestic, model or wild animal. Within these three animal groups we determine whether data collection is purely observational or instead the result of experimentation, which animal taxa are used, and which research questions are addressed. In addition, we use network analyses to determine unique and overlapping research foci for each animal group. Finally, we determine the extent that animal groups consider microbiota-host-environment interactions, by calculating the interdisciplinarity of studies within each group.

2.2.2 Data-mining the literature

A search for peer-reviewed articles on non-human gut microbiota published between the years 1911 and 2016 was performed in Web of Science® and PubMed. Search terms were 'microbi*' AND 'gut' OR other gut-related terms ('anal' OR 'anus' OR 'caec*' OR 'cec*' OR 'cloac*' OR 'colon' OR 'duoden*' OR 'faec*' OR 'fec*' OR 'gastro*' OR 'ile*' OR 'intest*' OR 'jejun*' OR 'rect*' OR 'rum*' OR 'stomach'). The search excluded common irrelevant terms ('ferment*', 'microbiol*', 'reactor*', 'review*', 'vitro'), and those related to humans ('child*', 'human*', 'infan*', 'men', 'paedi*', 'patient*'). All abstracts of the resulting 3,095 articles were reviewed manually and 1,419 were found to characterise the microbiota of the non-human animal gut (either the entire digestive tract, one or more sections, and/or faeces). A sub-set of 650 studies (November 2009 – July 2016) were randomly selected for analysis based on corresponding randomly generated numbers from all studies (Figure 2.1, Appendix A.1, Table A.1.1). Firstly, we categorised each study as focussing on animal species that were: 'domestic' (livestock and companion animals), 'model' (studied to provide insight into the microbiota of other organisms), or 'wild' (free-living or undomesticated animal species studied in their natural habitat or captivity). For each publication we noted whether data were 'observational', i.e., purely descriptive, or the result of a 'perturbation', i.e., a treatment was applied, such as a probiotic. We categorised the focal taxon for each study as mammal, bird, fish, reptile, amphibian, insect or non-insect invertebrate. Finally, 36 broad lines of enquiry ('research questions') were identified and quantified within each of the three animal groups (Figure 2.1, Appendix A.1, Table A.1.1).

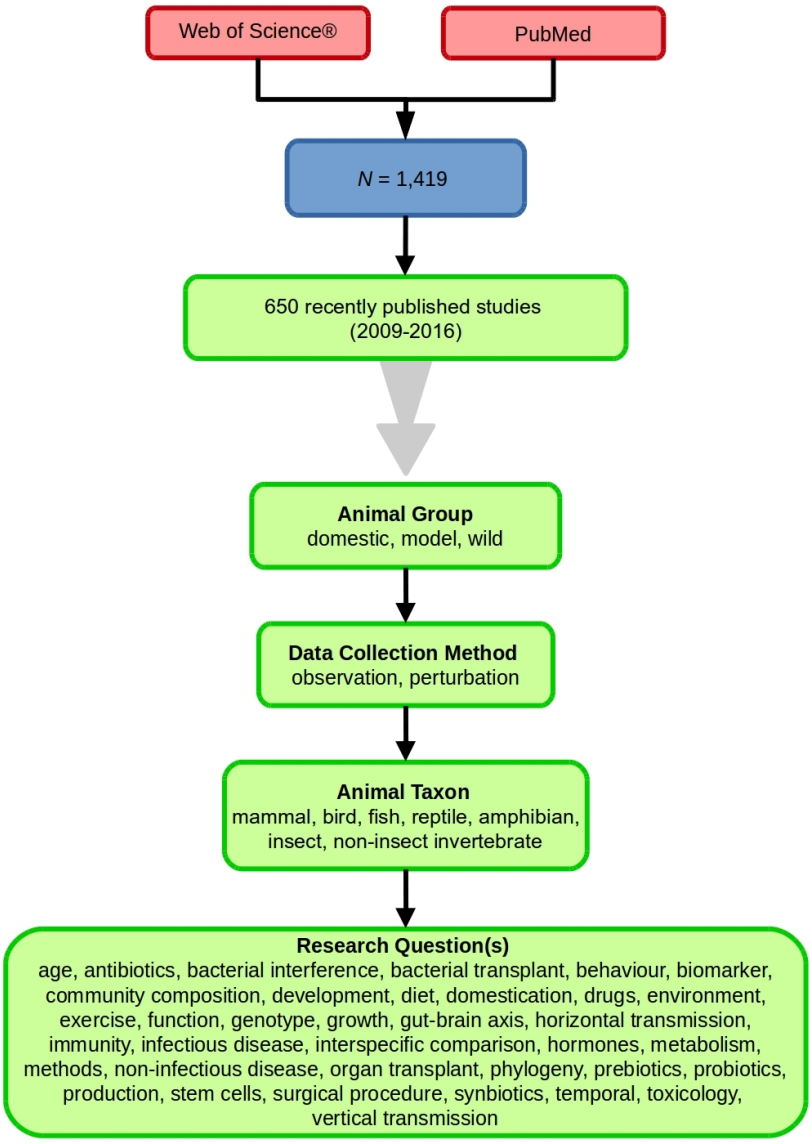

Figure 2.1: Work flow for categorising gut microbiota studies on non-human animals following searches in Web of Science® and PubMed. Of the 1,419 relevant articles identified, 650 recently published studies (2009-2016) were categorised into one of three animal groups (domestic, model or wild animals). Data collection method, animal taxon and research question(s) addressed were determined for each study.

2.2.3 What is driving animal microbiota studies?

The 650 publications reviewed here were dominated by studies on domestic animals (48.2%), followed by model animals (37.5%), while wild animal studies were comparatively few (14.3%;

Table 2.1). Perturbation is crucial to understand how a system functions, as exemplified by classic ecological experiments (Paine, 1966), and we found that it was used heavily, as opposed to observational data, in domestic studies (81.1%; Table 2.1). Likewise, perturbation was frequent in model studies (87.7%), but was rarely used in wild animals (20.4%), where instead observational data (79.6%) were favoured. All of the reviewed studies focussed on the bacterial communities of the microbiota, and of these, 12.5% studies also characterised at least one other microbial community; archaea (8.8%), fungi (4.3%), protozoa (2.8%) and/or viruses (0.6%; Appendix A.1, Table A.1.1). Just over half (54.3%) of studies that investigated the non-bacterial microbiota used perturbation, the remaining half being observational, and investigated domestic animals (53.1%), followed by wild (32.1%) and model (14.8%) animals.

In domestic animals, perturbation was used with the aim of improving animal productivity (29.7%), for example by administering probiotics (16.3%, e.g., Ahmed *et al.*, 2014) or prebiotics (6.4%, e.g., Hoseinifar *et al.*, 2014; Figure 2.2). In model animals perturbation was used to determine interactions between gut microbiota and host health, e.g., the role of microbiota in eliciting an immune response ('immunity'; 36.6%; e.g., Brinkman *et al.*, 2011) for forward translation to humans. For model animals, perturbation also included therapeutics, such as antibiotics (13.5%; e.g. Carvalho *et al.*, 2012), and more rarely, organ transplants (1.2%; Li *et al.*, 2011) and other surgical procedures (0.8%; Devine *et al.*, 2013, Figure 2.2). The few wild animal studies to use perturbation did so to understand system functions, e.g., by examining the effect of dietary treatments on microbiota of wild-caught giraffes, *Giraffa camelopardalis*, as a means to understand microbial symbioses (Roggenbuck *et al.*, 2014). Instead, observational data were the norm for wild animals in order to characterise 'natural' microbiota structure and function, especially community composition (41.9%; Figure 2.2).

Although perturbation, under controlled conditions, is more straightforward in domestic and model animals, thus facilitating treatment comparisons and reducing confounding factors such as genetic variation and diet, the complex combination of factors that influence microbiota are unlikely to be understood by looking at laboratory animals alone (McGuire *et al.*, 2008; Amato, 2013). Standardisation may appear logical to obtain less noisy data, but it does not reflect the human condition, where such identical factors are not experienced throughout life nor between individuals, and risks, what Ronald Fisher stated as "*(supplying) direct information only in respect of the narrow range of conditions achieved by standardisation*" (Fisher, 1937). It would appear that wild animals could provide an opportunity not only to examine natural gut microbiota function, but to extend observations to incorporate understanding of complex multidirectional microbiota-host-environment interactions that they are subject to. Already, other areas of traditionally animal-model dominated research, such as immunology, study and sometimes perturb wild model systems, giving rise to 'wild immunology' (Pedersen and Babayan, 2011), and it could be timely for microbiota research to follow suit. Consequently, the obvious progression of wild studies is to understand how 'natural' microbiota responds to perturbation as a model for humans and other species, and to determine directionality of microbiota-host-environment interactions (Gordon, 2012). Difficulties in doing so may be imposed, however, by legislation relating to scientific procedures on wild animals in any given country. In the UK, for example, the Animals Scientific Procedures Act 1986, must be complied with under Home Office regulations. In addition, species may be afforded protection from perturbation due to their international conservation status, for example, those appearing on the International Union for Conservation of Nature (IUCN) red list. Movement of samples between collaborators working on protected species may also be complex due to Convention on International Trade in Endangered Species (CITES)

regulations; permits are required for the translocation of samples from given species between countries. In a compromise between studying wild animals and meeting legal and logistical requirements, 40.9% of wild studies examined here used wild-caught (captured for purposes of study) or captive (e.g., from a zoo or research facility) 'wild' animals, with the remaining 59.1% investigating free-living, or a combination of free-living and captive animals. Even this level of compromise may significantly alter research outcomes, as it has consistently been found that wild animals exhibit a loss of natural microbes following captivity (Xenoulis *et al.*, 2010; Nelson *et al.*, 2013; Kohl and Dearing, 2014).

Table 2.1: The number of studies categorised into three animal study groups: domestic, model or wild, from 650 non-human animal gut microbiota studies, showing data collection methods (observation or perturbation) and network indices of three network graphs investigating research question interdisciplinarity and overlap.

Animal group	Data collection method		Number of nodes (*N*)	Maximum node size (*s*)	Maximum node degree* (*k*)	Maximum node strength† (*NS*)	Network density§ (*D*)	Mean betweenness centrality¤ (± SEM) (*BC*)
	Perturbation	Observation						
Domestic (48.2%)	256 (81.8%)	57 (18.2%)	27	Diet (158)	Diet (20)	Diet (175)	0.17	15.99 (± 3.41)
Model (37.5%)	214 (87.7%)	30 (12.3%)	34	Diet (95)	Immunity (23)	Immunity (164)	0.23	19.09 (± 3.99)
Wild (14.3%)	19 (20.4%)	74 (79.6%)	22	Community composition (39)	Diet (13)	Community composition (41)	0.08	12.19 (± 3.41)

** Node degree (k): The number of edges connected to a node, i.e., the number of research questions that co-occur.*

† Node strength (NS): The sum of the weighted edges connected to a node, i.e., the total number of separate co-occurrences of a research question and all others that it is connected to.

§Network density (D): The connections present in a network as a proportion of the total number of possible connections.

¤Mean betweenness centrality (BC): The mean shortest number of paths required to pass through each research question in the network, i.e., how well connected research questions are and thus interdisciplinarity of the whole network.

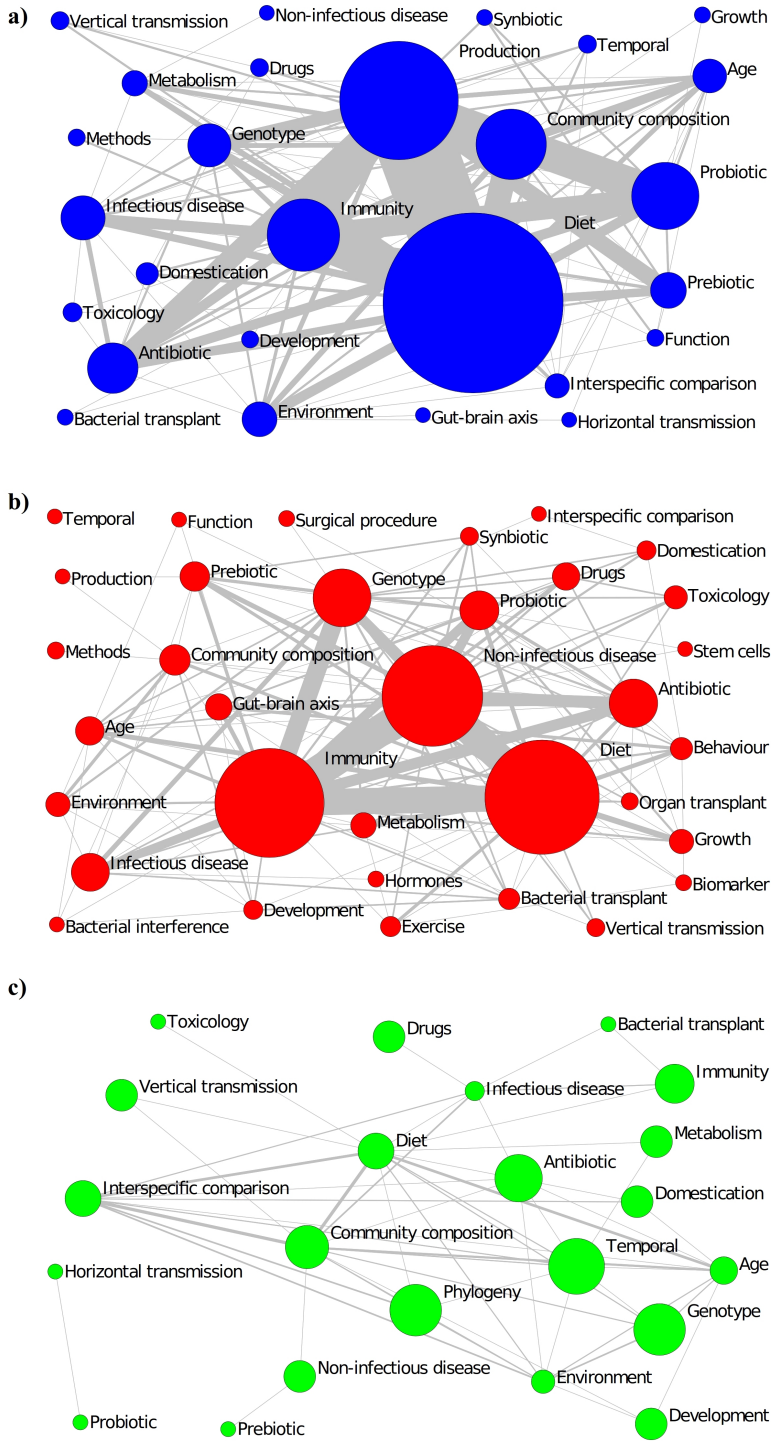

Figure 2.2: Network graphs illustrating the frequency of 36 research questions addressed by gut microbiota studies on a) domestic b) model and c) wild animals, and how frequently these questions co-occur within the 650 studies. Each node (circle) represents a research question, with diameter weighted by the number of studies. Edges (lines) connecting each node represent the co-occurrence of different research questions, with width weighted by the total number of co-occurrences.

2.2.4 How taxonomically diverse are animal microbiota studies?

Domestic and model studies were composed of similar taxonomic groups (predominantly vertebrates, i.e., mammals, birds and fish, in 97.1% and 93.0% of studies respectively), but the opposite was true of wild studies, which predominantly focussed on invertebrates (52.2%; Figure 2.3). Domestic animals that have large farmed populations in economically developed regions were most studied; i.e., pigs, cattle (49.7% and 28.7% of mammals respectively), and chickens (80.5% of birds; Figure 2.3). Species from all six taxonomic categories have been exploited as models, but model studies mostly focused on laboratory mice (70.2% mammals) or rats (23.3% mammals; Figure 2.3), in part because the dominant bacterial phyla in the rodent and human gut are similar - *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Spor *et al.*, 2011).

Laboratory model rodent studies have been fundamental for progressing our understanding of microbiota function and modulation, for example rats have demonstrated microbiota may be used as a biomarker to predict liver transplant rejection (Ren *et al.*, 2013). However, extrapolating data from laboratory animals to other species (including humans) has limitations, e.g., similarities in microbiota between rodents and humans are reduced beyond the phyla level (Spor *et al.*, 2011; Nguyen *et al.*, 2015). In addition, laboratory animals have a highly inbred genetic background (Hufeldt *et al.*, 2010), and are exposed to very different conditions to those experienced by humans and wild animals, but which influence microbiota, e.g., captive rearing (Zeng *et al.*, 2012), and constant extrinsic factors such as diet and housing conditions (Le Floc'h *et al.*, 2014). Indeed, the disparity between laboratory animals and humans is believed to be a major contributing factor towards attrition; whereby drug trials are successful in laboratory animals but later fail in human trials (Garner, 2014), and this same lack of successful forward translation is likely to also occur in microbiota research. As such, there appears to be a niche for utilising wild

rodents as model organisms: wild rodents are physiologically and genetically similar to those already used and understood in the laboratory (Pedersen and Babayan, 2011), but host an intact and diverse gut microbiota (Amato, 2013). Microbiota studies, however, on wild mammals are currently relatively uncommon (30.6%) and include species not related to those traditionally used as model organisms e.g., arctic ground squirrels (*Urocitellus parryii*) have been studied to monitor temporal changes in microbiota composition (Stevenson *et al.*, 2014). Instead, wild studies focussed on insects (42.5%), and although wild insects such as *Drosophila,* whose simple microbiota has provided insight into host-microbe interactions, could be developed as a model system (Chandler *et al.*, 2011), studies were instead driven by the potential for microbiota manipulation to be used in biocontrol. As such, wild insect studies were mainly focussed on agricultural pests and vectors of pathogens e.g., bee (23.4%), termite (22.1%) and mosquito species (13.0%; Figure 2.3). These, and similar studies, have suggested that removal of important symbiotic bacteria responsible for lignocellulose digestion could be used to control crop pests (Schloss *et al.*, 2006), and probiotics may be used to control vector-borne pathogens such as *Plasmodium* (malaria) in mosquitoes, since bacteria can stimulate an up-regulation of immunity genes that reduce *Plasmodium* acquisition (Dong *et al.*, 2009; Boissière *et al.*, 2012).

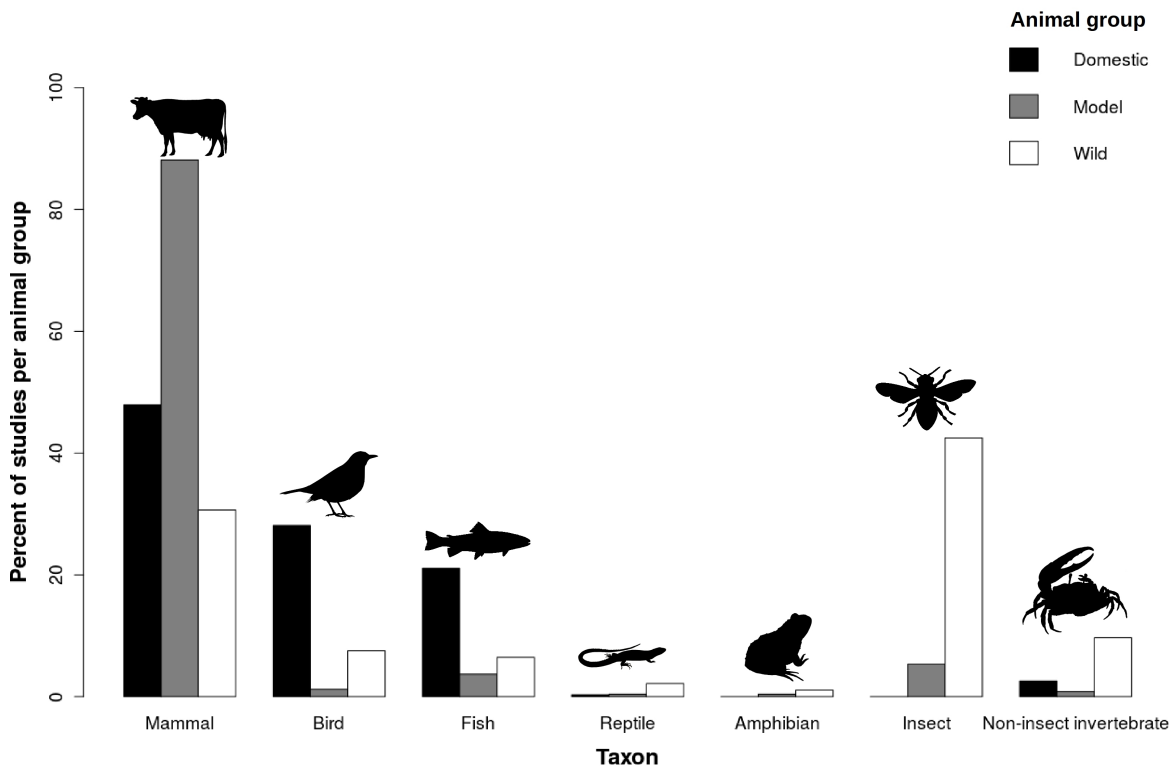

Figure 2.3: The percentage of gut microbiota studies within three animal groups; domestic (black), model (grey) or wild (white), investigating different animal taxa. For each animal group the combined percentage of studies across all taxa equate to 100% of studies for that group.

2.2.5 Using network analyses to visualise and quantify the research landscape

To visualise research foci and interdisciplinarity, network graphs were constructed for domestic, model and wild animal studies based on research questions. A network graph consists of nodes linked by edges; in this case, a node represented one of the 36 research questions identified, and the edges the co-occurrence of those questions within a scientific paper(s). Each network was constructed from an n by n symmetrical adjacency matrix; composed of a corresponding row and column for every node, where entries indicated links between two nodes (i, j). Edges were non-directed, i.e., a link between the nodes i, j had the same value as j, i. Node size (s) was weighted according to the total number of studies addressing that question, and edge width was weighted by the number of studies in which the two research questions co-occurred (Figure 2.2).

2.2.6 What are the research foci of animal microbiota?

To quantify and compare the foci of research questions between animal groups, we calculated a series of network metrics. Node size (*s*), or the number of studies investigating any given question depicts how common a question is; node degree (*k*) represents the number of edges connected to a question, thus its importance in forging links between disciplines, and node strength (*NS*) is the sum of weighted connections to a question, hence how core the question is to the research.

'Diet' was consistently a question of focus in all three animal groups (Table 2.1), but its research associations differed. In domestic animals 'Diet' was most commonly studied (*s* = 158), created the most links to other questions (*k* = 20) and did so frequently (*NS* = 175, Table 2.1). Thus, diet was fundamental and at the core of this research; often as a means to manipulate animal health via the microbiota, particularly to increase animal production (38.0% domestic diet studies; Figure 2.2). 'Diet' was also most frequently studied in model animals (*s* = 95), but with respect to host health and disease: 34.7% of such studies used diet specifically to treat or simulate non-infectious diseases such as obesity (Esposito et al., 2015) and diabetes (Prajapati *et al.*, 2015; Figure 2.2). Despite its popularity 'diet' was not the most integrated or interdisciplinary question in the network, but 'immunity' was (*k* = 23 and *NS* = 164; Table 2.1), highlighting the importance of the shared relationship between microbiota and immunity, and how it consequently affects many other aspects of health (Round and Mazmanian 2009). In contrast 'community composition' was most studied (*k* = 13) and embedded (*NS* = 41) within wild studies, but 'diet' was key to creating research links between questions (*s* = 39, Table 2.1). This link results from the fact that wild studies focus on microbiota structure (e.g., Delsuc et al. 2014), and suggests that we are currently acquiring more basal knowledge on wild animal microbiota. In addition, only 25.9% of wild

animal 'diet' studies used perturbations, with the remaining 74.1% observing microbiota composition under a 'natural' diet (33.3%; Figure 2.2). Given that 72% of emerging zoonotic pathogens are transmitted to humans from wildlife (Jones et al. 2008), and microbiota and immunity are strongly interlinked (Round and Mazmanian 2009), determining how microbiota interacts with host immunity and/or infectious disease (currently only 17.9% and 9.3% in domestic animals which have frequent contact with humans, and 3.2% and 10.8% of wild studies, respectively) deserves further consideration.

2.2.7 Do animal microbiota studies take an interdisciplinary approach?

Animal microbiota studies with a single research focus have provided important basal knowledge on microbial composition and function e.g. in-depth analyses of microbiota community composition in laboratory mice has revealed that the intestinal crypts, which harbour gut stem cells, also accommodate a niche microbial community (Pédron *et al.*, 2012). Likewise, there is also great value in an interdisciplinary approach in which multiple factors are studied simultaneously, and can aid in progressing knowledge and teasing apart complex and multidirectional host-microbiota-environment interactions (Gordon, 2012). We quantified the 'interdisciplinarity' of each group by measuring the mean 'betweenness centrality' (*BC*) of each network: *BC* indicates how closely associated all questions are in relation to each other, and is the number of shortest paths required to pass through each question to connect it to all other questions; larger values indicate questions are more closely associated (Leydesdorff, 2007). Network density (*D*), indicates the level at which interdisciplinarity has been exploited in each group, calculated as a proportion of the total number of possible connections, whereby 0 = no connections present and 1 = all possible connections are present and maximum interdisciplinarity has been reached. Network analyses were conducted using the igraph package in R v. i386 3.0.3

(Csardi and Nepusz, 2006).

Model studies exploited the ability to take an interdisciplinary approach the most, with the highest proportion of possible links between questions (D = 0.23), followed by domestic (D = 0.17) and wild (D = 0.08) studies (Table 2.1). In addition, research questions in model studies were more closely associated, directly or indirectly, with one another, (mean BC = 19.09 ± 3.99), than in domestic (BC = 15.99 ± 3.41) or wild (BC = 12.19 ± 3.41) studies (Table 2.1). The comparatively high interdisciplinarity of model studies reflects the large range of questions addressed (N = 34), compared to the domestic (N = 27) and wild (N = 22) groups, and the motivation of many model studies to improve medical treatments which often requires an interdisciplinary approach to monitor the range of subsequent effects on health (e.g., to investigate the associations between organ transplantation, non-infectious disease, immunity and microbiota; Xie *et al.*, 2014). Conversely, wild studies were least integrated and interdisciplinary; questions were addressed more independently of one another. However, this group did address a unique research question: 'phylogeny' – and how phylogeny is driven across species by gut microbiota and diet, and *vice versa*; for example, myrmecophagous mammals from different evolutionary lineages exhibit striking convergence with respect to gut microbial composition, driven by dietary adaptations (Delsuc *et al.*, 2014).

While the more focussed approach of wild animal research has allowed us to assemble fundamental microbiota knowledge, it has been argued that an interdisciplinary approach is necessary to progress research on basic and applied gut microbiota (Gordon, 2012). We predict that the interdisciplinarity of wild animal studies will increase as they are adopted in microbiota research, particularly if done so as model organisms. Indeed, the first interdisciplinary microbiota

studies using wild populations provide interesting insight into the interactions between host, microbiota and environment. For example, parasitic helminths infecting the gut have up- and down-stream effects on microbiota composition (Kreisinger *et al.*, 2015; Maurice *et al.*, 2015) and seasonal variation in wild rodent microbiota is largely driven by changes in food availability (Maurice *et al.*, 2015).

2.2.8 Conclusion and outlooks

Although more than 10% of studies investigated the microbial community of non-bacterial species in addition to the bacterial component of the microbiota, of these only 0.6% studies investigated the virome, despite evidence that viruses bestow a number of functional traits to bacteria (Ogilvie and Jones, 2015). Complementary studies that simultaneously investigate multiple components of the gut biome are likely to shed light on microbiota composition and functionality (see for example, Glendinning *et al.*, 2014). We demonstrate that most animal gut microbiota studies are driven by economic (domestic animals) or human health (model animals) issues, although more microbiota studies on immunity and/or infectious disease in domestic animals could benefit both livestock and humans in close proximity to them. There are, however, well-founded concerns regarding the limitations of laboratory animals as model organisms, as highlighted by attrition (Fisher, 1937; Garner, 2014). In 2013 the former director of the NIH, Prof. Elias Zerhouni, stated that "*We have moved away from studying human disease in humans*" (NIH Record: http://bit.ly/2f5UpII), arguing that we should "*....refocus and adapt new methodologies for use in humans to understand disease biology in humans*"; raising interesting issues about the use of animal models, including in microbiota research, and whether it is scientifically legitimate to forward translate our findings to humans. This does not mean that we should not use animal models, but rather that we should consider changing the way in which we study them, so that they

may more accurately represent human inter-individuality. The intact gut biomes of wild species that experience inter-individual and environmental variation more similar to humans than their laboratory counterparts, rendering the results more 'realistic', could form the basis of more relevant models to study microbiota. However, field experiments would need to be carefully designed to provide statistical power in the face of extensive variation (e.g., controlling for genetic background, diet, sex, etc.). Under some circumstances, manipulation of microbiota in wildlife is not possible (e.g., for rare, elusive or protected species). In these cases, development of mathematical and/or statistical models to assign directionality to observational data could be beneficial. Examples of applications in other fields include, identifying interactions between immune components using network theory (Thakar *et al.*, 2012), and determining interspecific interactions among an unperturbed community of gut parasites, using generalised linear mixed models (Fenton *et al.*, 2010). Studies on wild animals are currently comparatively few, and generally aim to characterise natural microbiota, combining few disciplines. However, we expect interdisciplinarity to increase in wild animals should they be developed as model systems.

2.3 Author Acknowledgements

The manuscript resulting from this chapter is currently in press in The ISME Journal and is authored by the following:

Emily L. Pascoe, Heidi C. Hauffe (Fondazione Edmund Mach), Julian R. Marchesi (Cardiff University and Imperial College), Sarah E. Perkins (Cardiff University and Fondazione Edmund Mach).

Chapter 3

Does disruption of the helminth community with anthelmintic affect the gut microbiota?

"To expect the world to receive a new truth, or even an old truth, without challenging it, is to look for one of those miracles which do not occur."

Alfred R. Wallace

3.1 Abstract

Anthelmintics are widely administered to humans, livestock, and companion animals in an attempt to control helminth infection. However, it is largely unknown whether or not there are knock-on effects associated with perturbation of the helminth community on other elements of the gut ecosystem, namely the microbiota. Here, anthelmintic (ivermectin) and a sham control (ultra-pure water) were administered to wild, *Apodemus flavicollis* harbouring natural helminth infections of the gut. The diversity, composition and OTU abundances of gut and faecal microbiota were recorded pre- and post-anthelmintic treatment. In addition, the efficacy of anthelmintic treatment was assessed by monitoring helminth abundance and fecundity pre- and post-treatment. Gut microbiota did not show significant taxonomical differences in composition between pre- and post-treatment individuals, but faecal microbiota did (Bray Curtis: d.f. = 52, F = 1.81, p <0.01; weighted UniFrac: d.f. = 52, F = 3.13, p <0.01). In addition, bacterial OTUs did not exhibit significant differences in abundance in the small intestine or colon after anthelmintic treatment, but did in the caecum, and faeces, and when whole gut microbiota were considered. There were substantial, but not significant, decreases in helminth abundance (95.4% overall reduction) and fecundity (46.7% and 69.5% reduction for *Heligmosomoides polygyrus* and *Hymenolepis* spp. respectively) associated with anthelmintic treatment. The results demonstrate that gut microbiota composition is resilient to perturbation of the helminth community, and is largely unaffected by the chemical ivermectin, but faecal microbiota is affected by anthelmintic treatment. It is possible that the changes in faecal microbiota associated with anthelmintic are a result of changes in host immunity following reduction in helminth infection. Given that many helminth species undergo development in host faeces, and faecal microbiota may provide an extension of the host immune phenotype against helminth resistance, the significant changes in faecal microbiota following anthelmintic treatment may affect helminth development and deserve further study.

3.2 Introduction

Billions of humans, as well as wildlife and livestock, harbour parasitic helminth infections in the gut (Morgan *et al.*, 2004; Hotez *et al.*, 2008; Lello *et al.*, 2013). Helminth infections can be asymptomatic (Checkley *et al.*, 2010), but can also lead to malnutrition, anaemia, reduced fecundity and other health issues (Shetty, 2010; Sutherland and Scott, 2010). As a result, helminth infections can have significant economic consequences; for example, in the United States of America the annual economic loss associated with nematode infection of sheep alone has been estimated at USD 42 million (Waller, 2006). Humans in westernised countries have access to flushing toilets that interrupt the life-cycle of many helminth species and prevent infection (Bilbo *et al.*, 2011), however, these are not currently accessible to the worldwide human population. Instead, widespread treatment with broad-spectrum anthelmintics is often employed to control helminth abundances in livestock, companion animals (Vlassoff *et al.*, 2001) and humans (Vercruysse *et al.*, 2012). In the event of mass drug administrations, individuals are frequently treated with anthelmintic indiscriminately and without evidence that all individuals are infected (Truscott *et al.*, 2015). In addition, many anthelmintic products are available 'over-the-counter' and so can be used inappropriately and without professional medical advice (Nielsen, 2009), contributing to widespread resistance of helminths to many of these pharmaceutical treatments (Wolstenholme *et al.*, 2004). Furthermore, we do not currently know if and how anthelmintic affects other components of the gut biome, and it is timely to understand these wider implications of treatment.

Helminths share the gut biome with the microbiota, the microbial community which includes bacteria, viruses and archaea. Helminths and microbiota share a long evolutionary history within the gut and therefore, like other organismal communities, interact with, and affect one another

(Glendinning *et al.*, 2014). Helminth infection can be associated with changes in microbial composition, which can occur in microbiota at the site of infection but also up- and downstream of this location (Cebra, 1999; Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Rausch *et al.*, 2013; Kreisinger *et al.*, 2015). For example, *Hymenolepis* species, which normally infect the small intestine, have been associated with microbiota variation in the host stomach (Kreisinger *et al.*, 2015), while an infection of the small intestinal nematode *H. polygyrus bakeri* induces microbial changes in the caecum and colon (Rausch *et al.*, 2013). It is not conclusively known how parasite infection influences microbiota, but a variety of factors have been proposed, including the secretion of bacterial growth inhibitors by some helminths (Hewitson *et al.*, 2009; Ditgen *et al.*, 2014), manipulation directly by the parasite to optimise conditions for helminth viability (Reynolds *et al.*, 2014), and/or three-way interactions between the microbiota, macrobiota and host immune system (Glendinning *et al.*, 2014). However, it is currently unknown if the changes in host microbiota associated with helminth infection can be reversed or altered when the helminth community is perturbed. Seminal papers in ecology have demonstrated that manipulating a system is crucial to understanding how its components interact (Paine, 1966). As such, perturbing the helminth community and monitoring the subsequent effects on the microbial community can help to determine the nature of helminth-microbiota interactions.

While there are numerous studies that perturb the helminth community by experimental infection of the host (e.g., Walk *et al.*, 2010; Li *et al.*, 2012; Rausch *et al.*, 2013; Reynolds *et al.*, 2014), to date only three studies have investigated the effects on microbiota of removing or reducing helminth infection (Cooper *et al.*, 2013; Sirois, 2013; Houlden *et al.*, 2015). Results are not consistent between these three studies, and range from the observation that microbiota can revert to a composition more similar to that of non-infected individuals following anthelmintic treatment

(Houlden *et al.*, 2015), to no detection of significant effects of anthelmintic (Cooper *et al.*, 2013). However, each study did administer anthelmintic to hosts harbouring an infection of a single helminth species (Cooper *et al.*, 2013; Houlden *et al.*, 2015; note, Sirois 2013 did not quantify helminth diversity or abundance), thus did not take into account the complexities of synergistic and antagonistic interactions that occur between coinfecting helminth species (Lello *et al.*, 2004; Telfer *et al.*, 2010), which may also impact the microbiota. The current study aims to test if microbiota composition undergoes changes following treatment with the commonly used anthelmintic ivermectin, in wild rodents naturally infected with multiple helminth species. The microbiota diversity, composition and changes in OTU abundances were monitored pre- and post-treatment using both gut and faecal samples.

3.3 Materials and methods

3.3.1 Study area and small rodent sampling

Live-trapping of *Apodemus flavicollis* was conducted using Ugglan multi-capture traps (Ugglan Type 2; Grahnab, Sweden) arranged in four grids of 64 traps each (8×8), with a 10 m inter-trap interval. Two grids were established at the locality of Cavedine (45°59'10.6"N, 10°57'47.1"E and 45°58'30.8"N, 10°57'22.0"E), and two at Pietramurata (46°00'52.2"N, 10°55'27.7"E and 46°00'47.7"N, 10°55'40.7"E) in the Province of Trento (Italy). Each grid occupied woodland with similar vegetation composition and structure, and was situated at least 500 m from neighbouring grids to minimise inter-grid movement of animals. Trapping grids at each locality were randomly assigned to either anthelmintic or sham control treatment. Traps were baited with sunflower seeds and potato for two nights on a consecutive biweekly basis, at each locality, from mid-May to August 2014. Following this pre-treatment monitoring of microbiota and macrobiota, trapping

was conducted at both localities intensively for four nights on a weekly basis during the treatment (August) and post-treatment monitoring periods (end of August to September). Throughout the course of trapping, a total of 144 different individuals were captured, 54 from anthelmintic assigned grids and 90 from control assigned grids. However, some of these individuals were excluded from analyses as they were not re-captured following treatment; of the 144 mice, 55.6% were captured on more than one occasion; 53.7% in anthelmintic assigned grids and 64.8% in control assigned grids. Animal trapping and handling procedures were authorised by the Comitato Faunistico Provinciale della Provincia di Trento, prot. n. 595 issued on 04 May 2011.

Upon initial capture, each mouse was tagged with a subcutaneous passive integrated transponder (Trovan ID 100; Ghislandi and Ghislandi, Italy), so that each individual could be identified at subsequent captures. Body mass, sex and breeding status were recorded. Mice were regarded as juveniles if the pelage indicated that the post-juvenile moult had not yet occurred (Gurnell *et al.*, 1990), whilst adults were categorised according to breeding condition (descended testes for males and perforated vagina or pregnant for females; Gurnell *et al.*, 1990); individuals with adult pelage that were not in breeding condition were classified as sub-adults. Each week, faeces collected at first capture of an individual were collected for faecal egg count (FEC) analyses, using a standard McMaster technique with saturated NaCl flotation solution, and helminth eggs per gram of faeces (EPG) was calculated (after Dunn and Keymer, 1986). When an individual was captured more than once during a trapping week, subsequent faecal samples were collected for microbiota analyses, and were transported to the laboratory at 4°C, whereupon they were immediately frozen at -80°C until DNA extraction (see '*3.3.4 16S rRNA gene sequencing'* below). A total of 25 mice (3 untreated and 9 treated from the anthelmintic group, and 6 untreated and 7 treated from the control group) were randomly selected throughout the course of the experiment for gut microbiota

and adult helminth analyses, and thus were euthanised by overdose of isoflurane, followed by cervical dislocation.

3.3.2 Macrobiota manipulation

During an 18-day period in August 2014 all adult and sub-adult mice captured at each grid were administered up to three doses of the respective treatment, with a minimum of seven days between each dose. The anthelmintic treatment consisted of ivermectin (Ivomec; Merial, Merck Sharp & Dohme, Netherlands) diluted in ultra-pure water. The anthelmintic solution was vigorously vortexed for 10 minutes each day before use. The sham control consisted of a dose of ultra-pure water. Each treatment was administered using a curved gavage needle (18 G × 50 mm) at a dose of 2 ml/Kg (following manufacturer's instructions for Ivomec; and after Ostlind *et al.*, 1985, see also Pritchett and Johnston, 2002). Due to the vagaries of trapping wild animals, not every individual was captured three times/with a sufficient time interval between doses throughout the treatment period to receive the intended three doses of treatment: a total of 23 individuals were treated with anthelmintic (one dose $n = 3$, two doses $n = 9$, three doses $n = 11$), while due to differences in population density in the control group, 42 individuals were treated with the control sham gavage (one dose $n = 30$, two doses $n = 11$, three doses $n = 1$).

3.3.3 Analyses of gut samples

The 25 euthanised *A. flavicollis* were dissected under sterile conditions following methods in Kreisinger *et al.*, (2015). Briefly, the gut was washed in sterile Tris-buffered saline (TBS; Tris-NaCl; 50 mM Tris, 200 mM NaCl, pH8) and separated into four functional sections (stomach, small intestine, caecum, and colon). The luminal contents and membrane of each section were diluted with TBS and scanned for helminths at 10× magnification (Leica© MS5 microscope with

a Leica© CLS100 light attachment). After thoroughly scraping the gut membrane with tweezers under TBS to dislodge bacteria, the membrane and the TBS containing bacteria were collected with the rest of the luminal contents in a centrifugation tube. A bacterial pellet was obtained from the gut material by centrifugation (950 G for 10 minutes at 4°C, resulting supernatant 9,000 G for 15 minutes at 4°C. The membrane did not form part of the pellet during the second centrifugation and was discarded). The bacterial pellet was immediately stored at -80°C for future bacterial DNA analysis (see '*3.3.4 16S rRNA gene sequencing*' below).

3.3.4 16S rRNA gene sequencing

A total of 56 frozen faecal samples, which included at least one pre- and one post-treatment sample from any given individual, were sequenced for microbiota analyses; 37 samples from 15 individuals from the anthelmintic group, and 19 samples from 8 individuals from the control group. In addition, small intestine, caecum and colon samples from the 25 euthanised individuals were sequenced. The QIAmp DNA Stool Mini kit (Qiagen, Valencia, CA, USA) was used for total genomic DNA extraction from each luminal bacteria sample (small intestine, caecum and colon. The microbiota of the stomach was not analysed) and from faeces. In addition to the methods provided by the manufacturer for pathogen detection, a 2 minute homogenisation step at 30 Hz was performed to enhance bacterial cell lysis, using a Mixer Mill MM200 (Retsch GmbH, Haan, Germany) with 5 mm stainless steel beads (Qiagen, Valencia, CA, USA). Purity and quality of the recovered DNA were determined using a QIAxcel capillary electrophoresis system (Qiagen, Valencia, CA, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified using the 341F and 805R primers (see Appendix A.2, Figure A.2.1 for details on primer sequences, including degenerate nucleotides), and sequenced using a 2×300 bp kit on the Illumina MiSeq system (Illumina, San Diego, CA, USA). The PCR reactions were carried out in a total volume of

25 µl, containing 0.4 µM of each primer, 0.4 mM of dNTP (Promega, Madison, WI, USA), 1× FastStart reaction buffer (Roche Diagnostics GmbH, Mannheim, Germany), 1 mM of $MgCl_2$, 1.25 unit of FastStart HiFi Polymerase (Roche Diagnostics GmbH, Mannheim, Germany), and 12.5 ng of genomic DNA for each sample amplification. Thermal cycling was performed on a GeneAmp™ PCR System 9700 instrument (Thermo Fisher Scientific, Waltham, MA, USA) as follows: initial denaturation at 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 55°C for 45 seconds, 72°C for 1 minute 15 seconds, and a final extension at 72°C for 8 minutes. Negative controls for extraction and PCR reactions were included, and genomic DNA from Microbial Mock Community B (Staggered, Low Concentration), v5.2L (BEI Resources, Manassas, VA, USA) was included to assess the effect of data processing on observed community content. Dual indices and Illumina sequencing adapters were attached using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). The final library was cleaned, quantified, normalised and pooled in an equimolar way before sequencing on the Illumina MiSeq system (Illumina, San Diego, CA, USA) at the University of Trento, Trento, Italy. Sequencing was carried out following the manufacturer's recommendations.

3.3.5 Bioinformatic processing of 16S data

Sequences were merged, trimmed and filtered using MICCA software (version 1.5.0, Albanese *et al.*, 2015). Overlapping regions of the forward and reverse read sequences that differed by more than eight nucleotides or did not contain both the forward and reverse PCR primer sequences were discarded. The resulting, merged 16S fragments were discarded if they had an average expected error (AvgEE) greater than 0.1. Operational taxonomic units (OTUs) were assigned using a *de novo*, greedy strategy using a cut-off of 97% similarity, based on the VSEARCH clustering algorithm implemented in MICCA (Rognes *et al.*, 2016). Chimeric sequences were discarded.

Resulting representatives of each OTU were classified using the Ribosomal Database Project classifier (RDP classifier, version 2.12; Michigan State University [http://rdp.cme.msu.edu/]). Samples that had final read counts of less than 10,000 merged and quality-filtered reads were discarded. The resulting OTUs were analysed at the phylum and class level using Phyloseq version 1.16.2 (McMurdie and Holmes, 2013).

3.3.6. Statistical analyses of microbiota - diversity

Generalised linear mixed models (GLMMs) were used to assess whether there was a significant association between microbiota alpha diversity (inverse Simpson index) and anthelmintic treatment. Preliminary analyses indicated that data had insufficient power to include treatment interacting with treatment period (anthelmintic and control data pooled) as an explanatory variable, thus anthelmintic and control data were analysed in separate GLMMs; firstly a GLMM was used to test that there were no significant differences in helminth abundance, EPG, fecundity, female percentage and size in pre-treatment individuals between the anthelmintic and control group to ensure changes in post-treatment individuals were comparable. Once this assumption was confirmed separate GLMMs were run with alpha diversity of either the small intestine, caecum, colon, whole gut (small intestine, caecum and colon combined) or faeces as the response variable. Host sex, breeding status and treatment period (pre- or post-treatment) were explanatory variables. The identity code of the individual, geographical location (Cavedine or Pietramurata) and sampling month were each modelled as a nested random intercept for each model. A process of multi-model inference was used to compare all possible models using the R package ‘MuMIn’ (Bartoń) and the most parsimonious model was selected using a threshold of ΔAICc <2 (Burnham and Anderson, 2003).

3.3.7 Statistical analyses of microbiota - composition

A distance-based redundancy analysis (db-RDA; capscale function in R package *vegan*) was used to test for differences in microbiota composition associated with anthelmintic treatment, in the small intestine, caecum, colon, whole gut (small intestine, caecum and colon combined) or faeces. Ecological distances between microbiota communities from pre-treatment and post-treatment individuals (for both anthelmintic and control) were assessed using Bray–Curtis dissimilarities (i.e., compositional dissimilarity index that accounts for proportional differences of OTUs among samples) and weighted UniFrac dissimilarity matrices (which accounts both for proportional differences of OTUs and their phylogenetic relatedness; Lozupone and Knight, 2005). OTU tables were scaled before calculation of dissimilarity matrices to achieve an even sequencing depth, corresponding to the minimal number of reads per sample in gut sections or faeces that were included in a given analysis. Significance was assessed using permutation-based marginal tests.

3.3.8 Statistical analyses of microbiota - OTU abundances

To determine how OTU abundances varied following anthelmintic treatment, OTUs with a differential abundance (i.e., number of reads corrected for sequencing depth) between pre- and post-treatment individuals in the small intestine, caecum, colon, the whole gut and in faeces were first identified, using an approach based on generalised linear models with negative binomial errors implemented in the DESeq2 package (Anders and Huber, 2010). These analyses were run using the default pipeline set-up in DESeq2, and significance values (p >0.05) were derived using likelihood-ratio tests.

3.3.9 Statistical analyses of helminth abundance and EPG

GLMMs were used to test for significant differences associated with anthelmintic treatment on

total helminth abundance (total number of helminths present, including zero values of uninfected hosts, as defined by Bush *et al.*, 1997) and abundance of *Heligmosomoides polygyrus* and *Hymenolepis* species. Due to a lack of power, differences in abundances of the other two species identified, *T. muris* and *S. frederici,* were not individually statistically analysed, but were included in total helminth abundance analyses; only a single *Trichuris muris* and 15 *Syphacia frederici* were present in a single individual in the population.

In addition, GLMMs were used to test for significant differences associated with anthelmintic treatment on total helminth EPG (here defined as the total number of helminth eggs present in faeces, including zero values of uninfected hosts), and EPG of *H. polygyrus* and *Hymenolepis* species. A total of 118 FEC measurements, which included at least one pre- and one post-treatment sample from any given individual (63 FECs from 10 individuals from the anthelmintic group and 55 FECs from 14 individuals from the control group) were used for statistical analyses of EPG. Due to a lack of statistical power, differences in *T. muris* and *S. frederici* EPG were not individually statistically analysed but were included in total EPG analyses; only one *Trichuris muris* egg and one *Syphacia frederici* egg were present in faeces from the anthelmintic group. Preliminary analyses indicated that data had insufficient power to include treatment interacting with treatment period (anthelmintic and control data pooled) as an explanatory variable, thus anthelmintic and control data were analysed in separate GLMMs; firstly a GLMM was used to test that there were no significant differences in helminth abundance, EPG, fecundity, female percentage and size in pre-treatment individuals between the anthelmintic and control group to ensure changes in post-treatment individuals were comparable. Once this assumption was confirmed the response variables were either helminth abundance or EPG. Host sex, host breeding status, host body mass and treatment period (pre- or post-treatment) were explanatory variables. In

addition, the model included the following two-way interaction terms as explanatory variables: all possible two-way interactions between host sex, host breeding status and host body mass. The identity code of the individual, geographical location (Cavedine or Pietramurata) and sampling month were all modelled as a nested random intercept for each model. For each GLMM, a process of multi-model inference was used to compare all possible models using the R package 'MuMIn' (Bartoń). The most parsimonious model was selected using a threshold of ΔAICc <2 (Burnham and Anderson, 2003). Statistical analysis used the package 'glmmADMB', version 8.3.3 (Fournier *et al.*, 2012; Skaug *et al.*, 2016).

3.4 Results

3.4.1 The effect of anthelmintic on gut and faecal microbiota diversity

Of the sequenced samples, reads from two faeces, one small intestine, one caecum and one colon samples were discarded as they did not meet the quality filtering criteria. The filtered microbiota dataset consisted of 2,639,407 high-quality reads from 126 samples (mean ± standard error = 20,948 ± 598 range = 10,363 – 49,083), within which 15 phyla were identified.

In the anthelmintic group alpha diversity of small intestine microbiota decreased from 28.7 ± 15.5 in pre-treatment individuals to 9.0 ± 4.7 in post-treatment individuals (d.f. = 6, Z = -1.70, p = 0.09), while in the control group alpha diversity of the small intestine significantly increased post-treatment (d.f. = 10, Z = 2.71, p <0.01; Figure 3.1). The alpha diversity of the caecum increased in both the anthelmintic and control group in post-treatment individuals, although not significantly (d.f. = 7, Z = -0.82, p = 0.41 and d.f. = 7, Z = 0.77, p = 0.44 respectively; Figure 3.1). Alpha diversity of the colon increased from 31.4 ± 13.0 to 32.1 ± 6.5 post- anthelmintic treatment, but

decreased in the control group (d.f. = 7, Z = 0.37, p = 0.71 and d.f. = 10, Z = -0.06, p = 0.96 respectively; Figure 3.1). In faeces, alpha diversity of microbiota decreased after anthelmintic treatment (d.f. = 32, Z = -1.83, p = 0.07) and in post- control treatment individuals (d.f. = 14, Z = 0.22, p = 0.82).

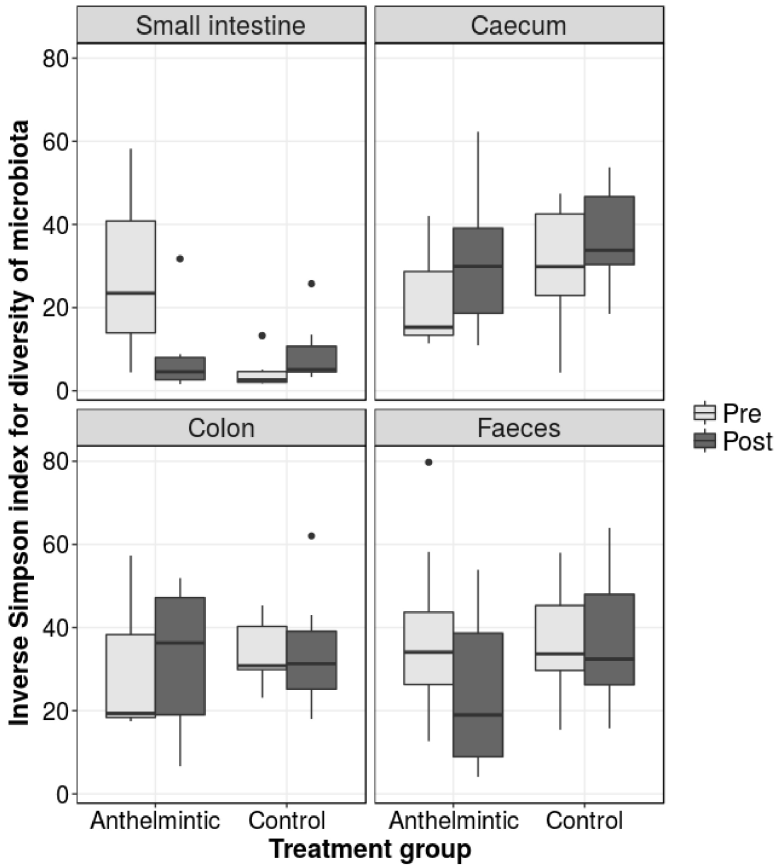

Figure 3.1: Inverse Simpson diversity index for alpha diversity of microbiota at three different sites within the gut (small intestine, caecum and colon), and faeces, for pre- and post-treatment individuals in an anthelmintic or control group. Boxes demonstrate the upper and lower quartiles of alpha diversity, with median alpha diversity indicated. Bars represent the minimum and maximum range of alpha diversity.

3.4.2 The effect of anthelmintic on gut and faecal microbiota composition

The majority of all 16S rRNA reads yielded from gut and faecal samples were from the phylum

Bacteroidetes (41.7%), followed by *Firmicutes* (40.6%) and *Proteobacteria* (10.6%). Of note, 18.0% of reads from small intestine samples were of the phylum *Tenericutes* (Figure 3.2). At the class level, 41.4% of reads were dominated by *Bacteroidia,* 33.5% by *Clostridia* and 6.7% by *Gammaproteobacteria*, whilst reads from the small intestine were also dominated by *Bacilli* (27.3%) and *Mollicutes* (17.9%; Figure 3.2).

The taxonomic composition of whole gut microbiota showed significant changes associated with anthelmintic treatment when Bray-Curtis (d.f. = 66, $F = 1.63$, $p<0.01$), but not weighted UniFrac (d.f. = 66, $F = 1.34$, $p = 0.19$) dissimilarity was considered (Figure 3.3). Treatment did not cause significant differences in the taxonomic composition of small intestine microbiota (Bray-Curtis: d.f. = 19, $F = 0.96$, $p = 0.55$; weighted UniFrac: d.f. = 19, $F = 0.80$, $p = 0.68$; Figure 3.4), nor in caecum microbiota (Bray-Curtis: d.f. = 20, $F = 1.00$, $p = 0.49$; weighted UniFrac: d.f. = 20, $F = 1.32$, $p = 0.12$; Figure 3.4). However, anthelmintic treatment did have a significant effect on colon microbiota composition, but only according to weighted UniFrac dissimilarity (Bray-Curtis: d.f. = 19, $F = 1.15$, $p = 0.13$; weighted UniFrac: d.f. = 19, $F = 2.34$, $p = 0.02$; Figure 3.4). In addition, taxonomic composition of faecal microbiota significantly differed following anthelmintic treatment (Bray-Curtis: d.f. = 52, $F = 1.81$, $p<0.01$; weighted UniFrac: d.f. = 52, $F = 3.13$, $p<0.01$; Figure 3.5).

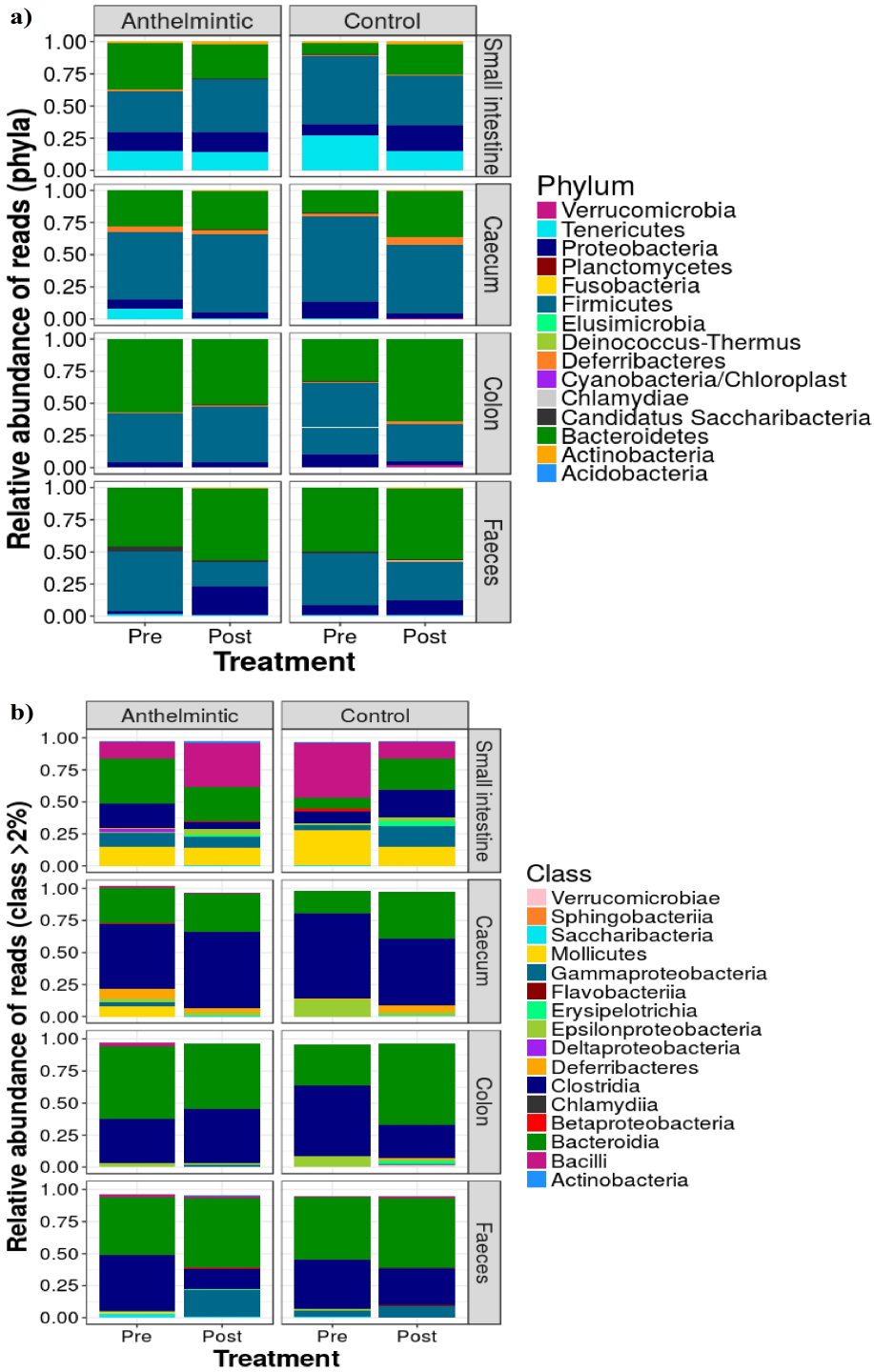

Figure 3.2: Mean relative abundance of bacterial a) phyla and b) classes (consisting >2% reads) present in the small intestine, caecum, colon and faeces of pre- and post-treatment mouse individuals in an anthelmintic or control group.

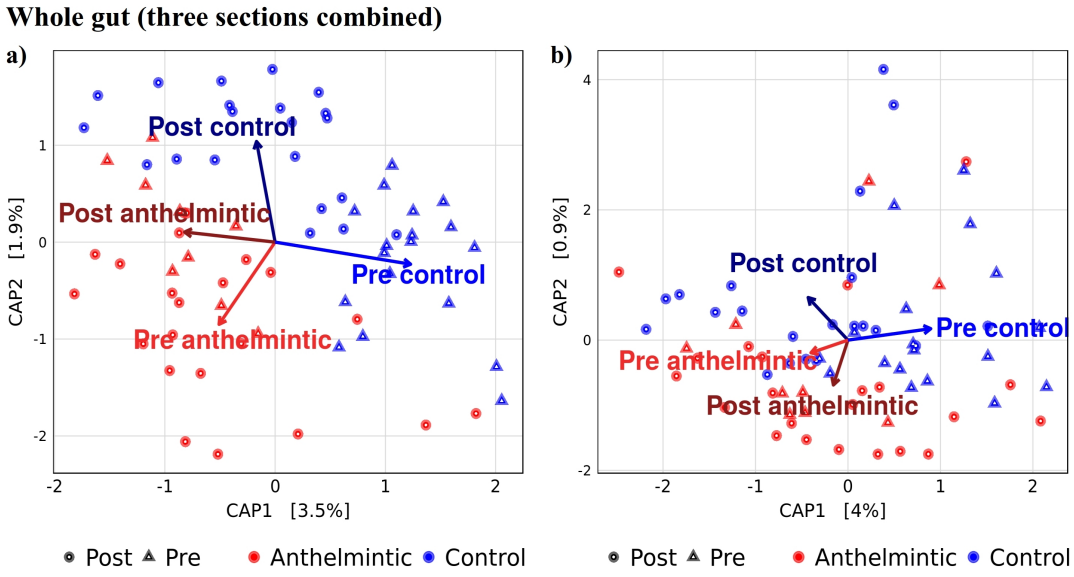

Figure 3.3: Ordination plots of divergence of microbiota taxonomic composition between samples of three gut sections (small intestine, caecum and colon) associated with either anthelmintic treatment or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation are shown. The length of the arrow indicates the relative importance of each treatment.

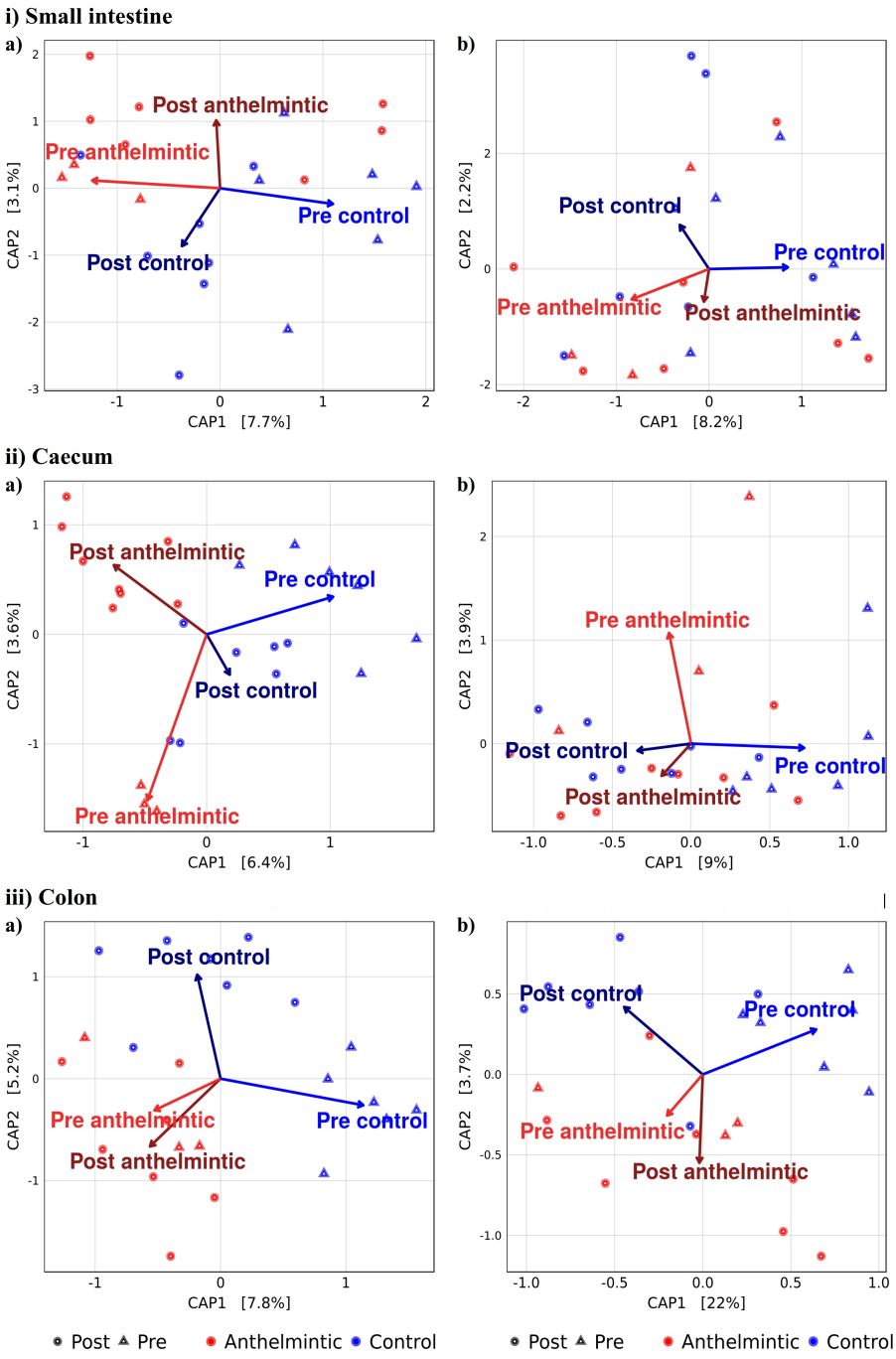

Figure 3.4: Ordination plots of divergence of microbiota taxonomic composition between i) small intestine, ii) caecum and iii) colon samples, associated with either anthelmintic treatment or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation are shown. The length of the arrow indicates the relative importance of each treatment.

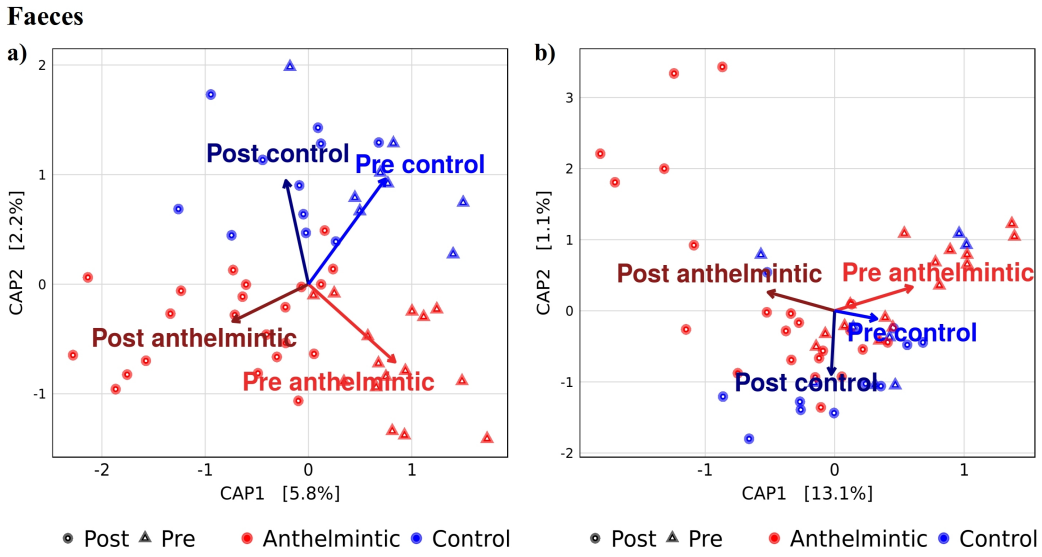

Figure 3.5: Ordination plots of divergence of microbiota taxonomic composition between faeces samples associated with either anthelmintic treatment or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation are shown. The length of the arrow indicates the relative importance of each treatment.

3.4.3 The effect of anthelmintic on gut and faecal microbiota OTU abundances

In microbiota of the whole gut (three gut sections combined), changes in OTU abundance associated with anthelmintic treatment were analogous to those seen in the post-treatment control group (Figure 3.6; see Appendix A.3 and tables therein for detailed statistics). For example, the increase of some OTUs in the classes *Clostridia, Deltaproteobacteria* and *Bacteroidia* in post-treatment individuals of both groups (Figure 3.6; see Appendix A.3, Table A.3.1 and A.3.2). In the caecum, anthelmintic treatment had an effect on abundance of OTUs from just two bacterial classes, both of which were significantly lower in abundance; *Clostridia* and *Mollicutes* (see Appendix A.3, Table A.3.4 and A.3.5). Microbiota of faeces, however, did show substantial changes in the abundance of OTUs post-anthelmintic treatment; OTUs from nine bacterial classes were significantly affected post- treatment, compared to just two bacterial classes (*Clostridia* and

Gammaproteobacteria which both showed similar patterns in abundance change as in the anthelmintic group) as observed post-treatment in the control group (Figure 3.6; see Appendix A.3, Table A.3.7 and A.3.8).

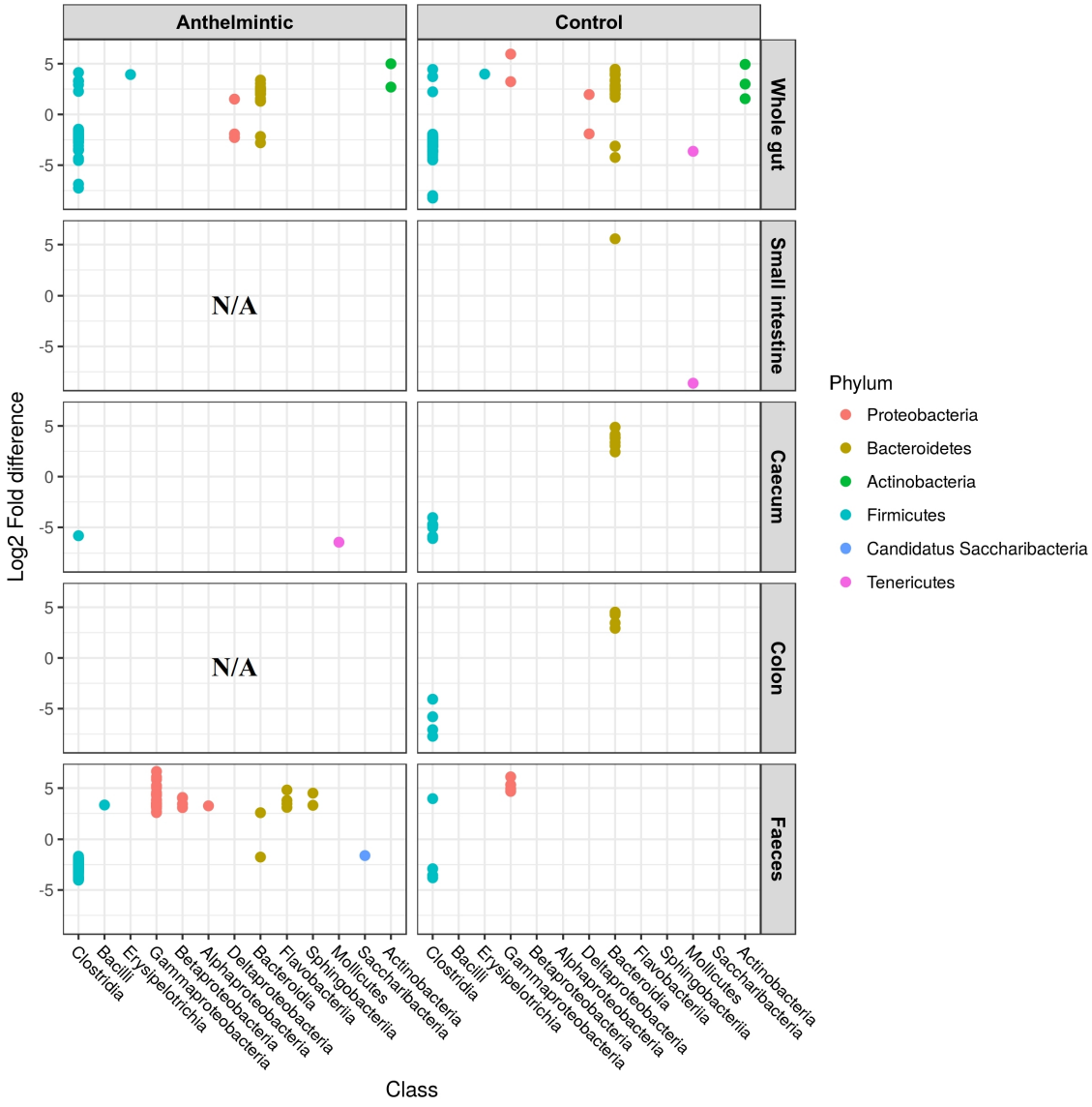

Figure 3.6: OTUs in the gut microbiota that were significantly different in abundance in post-treatment compared to pre-treatment individuals in an anthelmintic treatment and control group. Microbiota of the whole gut (three gut sections combined), small intestine, caecum, colon and faeces were analysed. OTUs were grouped by microbial class. Briefly, DESeq was used to identify significantly different (p <0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing pre- and post-treatment mice. N/A indicates gut sections in which no OTUs exhibited significant changes in abundance between pre- and post-treatment individuals.

3.4.4 The effect of anthelmintic on helminth abundance

To determine treatment efficacy, the effect of anthelmintic on helminth prevalence and abundance was tested. Prevalence of *H. polygyrus* and *Hymenolepis* was lower in post- compared to pre-anthelmintic treated individuals (Figure 3.7). In addition, anthelmintic treatment was associated with a non-significant reduction in helminth abundance; mean abundance was 95.4% lower post-treatment (153.0 ± 143.0 to 7.0 ± 1.4; d.f. = 9, Z = -1.59, p = 0.11), and 66.6% lower post-treatment in the control group (75.3 ± 43.9 to 25.1 ± 10.7; d.f. = 10, Z = -0.64, p = 0.52). Specifically, *H. polygyrus* abundance was 56.1% lower in post-treatment individuals in the anthelmintic group (6.3 ± 0.7 to 2.8 ± 0.8; d.f. = 10, Z = -1.07, p = 0.29) and 37.6% lower in the control group (from 11.7 ± 5.0 to 7.3 ± 1.6; d.f. = 8, Z = -0.84, p = 0.40; Figure 3.7 and 3.8). *Hymenolepis* spp. abundance was 97.2% lower in the anthelmintic group (141.7 ± 138.7 to 4 ± 1.4; d.f. = 8, Z = -2.13, p = 0.03), but 75.6% higher in the post-treatment individual from the control group (from 10.2 ± 4.8 to 17.9 ± 11.2; d.f. = 9, Z = 0.70, p = 0.49; Figure 3.7 and 3.8).

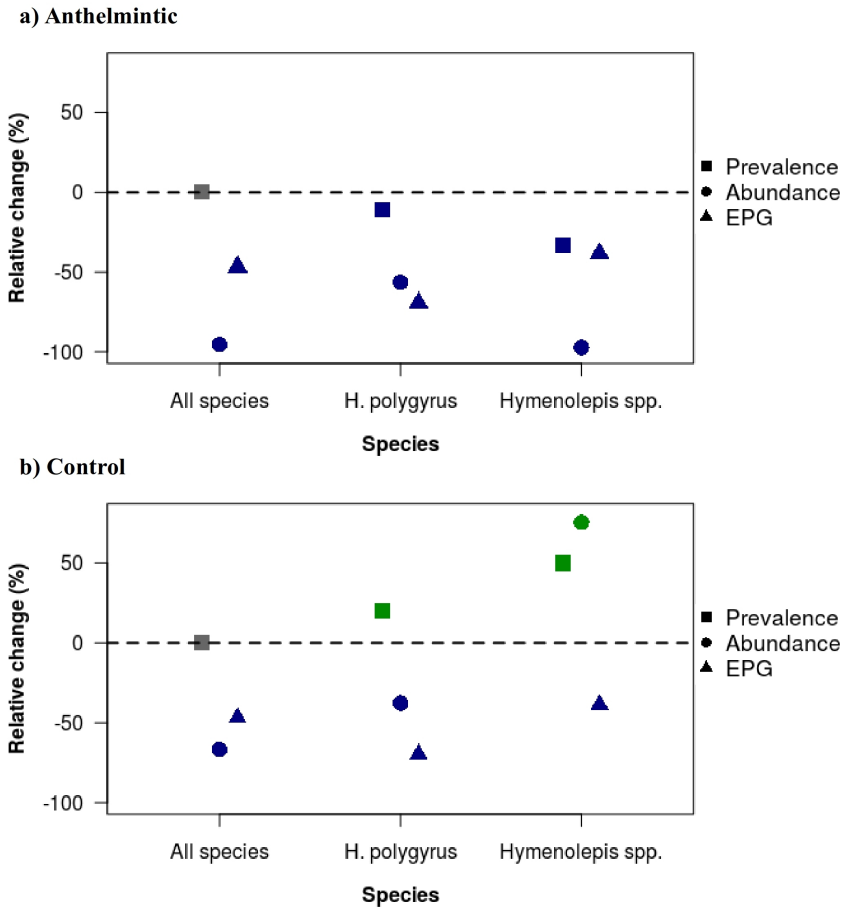

Figure 3.7: Relative changes (%) in helminth prevalence, abundance and eggs per gram (EPG) of faeces between pre- and post-treatment individuals in an a) anthelmintic and b) control group for all helminth species, *Heligmosomoides polygyrus* and *Hymenolepis* spp. Prevalence, abundance and EPG of other identified species were insufficient to perform statistical analyses. Blue data points indicate where there was a relative decrease, green indicates a relative increase and grey indicates where no change was observed between pre- and post-treatment individuals.

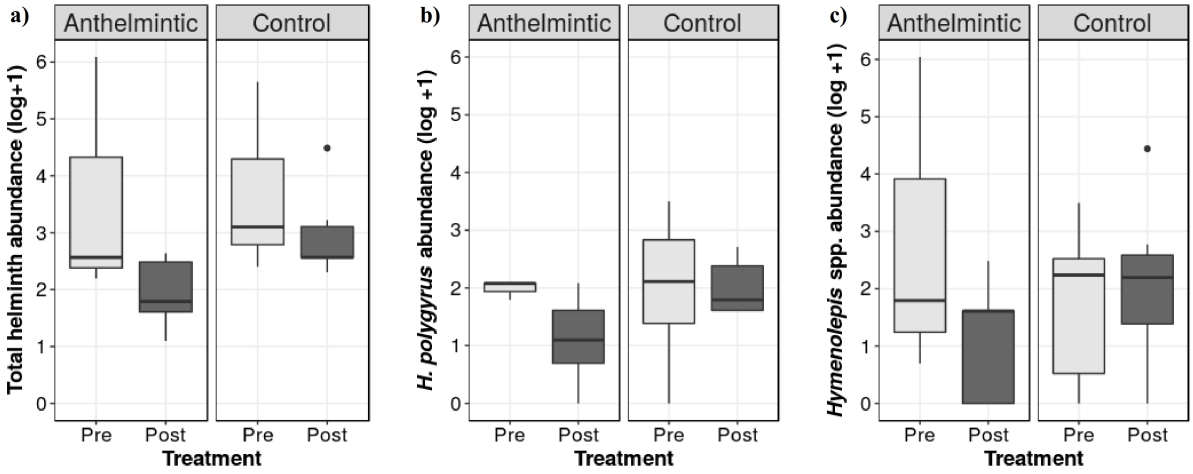

Figure 3.8: Abundance (log +1) of a) all helminth species, b) *Heligmosomoides polygyrus*, c) *Hymenolepis* spp. in individuals pre- and post-treatment for an anthelmintic treatment and control group. Abundance of other identified species were insufficient to perform statistical analyses.

3.4.5 The effect of anthelmintic on helminth fecundity

In the anthelmintic group, the mean helminth eggs per gram (EPG) of faeces decreased by 46.7% (1,076.7 ± 500.8 EPG to 574.1 ± 148.3 EPG) in post- compared to pre-treatment individuals, but not significantly so (d.f. = 58, Z = -0.35, p = 0.73), and in the control group helminth EPG increased post-treatment, by 49.0%, but not significantly so (from 546.5 ± 223.2 to 814.3 ± 246.8; d.f. = 50, Z = 0.75, p = 0.45). EPG of *H. polygyrus* exhibited a non-significant reduction of 69.5% between pre- and post-anthelmintic treatment (d.f. = 118, Z = -0.12, p = 0.90; Figure 3.7 and 3.9), and by 37.6% in the control group (d.f. = 8, Z = -0.84, p = 0.40). Mean EPG of *Hymenolepis* spp. also decreased (by 38.5%) in post- compared to pre-anthelmintic treatment (d.f. = 58, Z = -1.21, p = 0.23; Figure 3.7 and 3.9), but increased by 75.6% in the control group (d.f. = 9, Z = 0.70, p = 0.49).

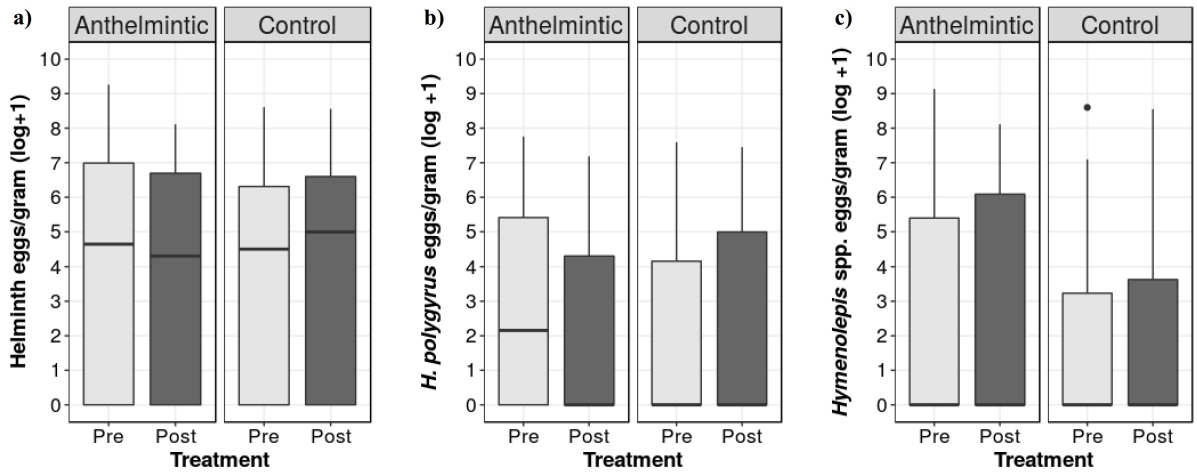

Figure 3.9: Helminth eggs per gram (EPG) of faeces (log +1) of a) all helminth species, b) *Heligmosomoides polygyrus*, c) *Hymenolepis* spp. in pre- and post-treatment for an anthelmintic treatment and control group. EPG of other identified species were insufficient to perform statistical analyses.

3.5 Discussion

Although the effect was not significant, anthelmintic treatment was associated with a general decrease in gut microbial diversity, with the exception of the caecum and colon, in which diversity increased (Figure 3.1). Anthelmintic treatment did have a significant effect on the taxonomic composition of microbiota, but only in the colon and faeces, and when all three gut sections where considered together (Figure 3.2 - 3.5). Anthelmintic had little affect on the abundance of bacteria; differences in OTU abundances between pre- and post-treatment individuals mirrored those seen between pre- and post-treatment individuals in the control group, or were non-existent (Figure 3.6, see Appendix A.3 for detailed statistics).

Previous studies on the effect of anthelmintic treatment on microbiota have yielded mixed results; one study saw microbiota of faeces from individuals experimentally infected with helminths

'restored' to a microbial community more similar to uninfected individuals (Houlden *et al.*, 2015). Such dramatic changes in microbiota composition were not observed in the current study. However, in the present study, all individuals were already naturally infected with at least one helminth species, and microbiota were not compared before and after infection, thus this comparison made by Houlden *et al.* (2015) could not be made. In addition, in the Houlden *et al.* (2015) study, helminth infection was experimental, and the model system was a laboratory rodent, which is unlikely to have represented the same complexities of microbiota and macrobiota interactions as represented by the wild, replete system studied here (Amato, 2013). Regardless, in the current study there were some significant changes in microbiota associated with anthelmintic treatment; there was a significant change in taxonomic composition of faeces (Figure 3.5), and OTUs from four phyla changed significantly in abundance in these samples (Figure 3.6). *Bacteroidetes* constituted one of these four phyla, all OTUs of which, barring one, increased post-anthelmintic treatment, while Houlden *et al.*, (2015) also observed increases in *Bacteroidetes* abundance (and diversity) following anthelmintic treatment. Interestingly, the contrary pattern was observed in horses treated with anthelmintic; the *Bacteroidetes: Firmicutes* ratio shifted such that *Bacteroidetes* relative abundance decreased, but *Firmicutes* increased (Sirois, 2013). Furthermore, no affect of anthelmintic treatment on microbiota composition was observed in naturally infected humans (Cooper *et al.*, 2013). The disparity in results between this study and the three others which have investigated the effect of anthelmintics on microbiota could be due to the comparison of such few publications, and may also be a result of variation between studies in the host species which was investigated, as well as the anthelmintic used (Cooper *et al.*, 2013; Sirois, 2013; Houlden *et al.*, 2015).

Given the widespread and often ungoverned use of anthelmintics in humans, livestock and

companion animals (Vlassoff *et al.*, 2001; Nielsen, 2009; Vercruysse *et al.*, 2012), the evidence that microbiota can remain largely stable following treatment provides reassuring evidence that anthelmintics have a minimal direct negative affect on the microbiota, particularly as the World Health Organisation has committed to increase the percentage of children treated with anthelmintic to 75% by 2020 in areas were helminth infection prevalence is greater than 20% (Truscott *et al.*, 2015). Although the avermectin family of anthelmintics (which includes ivermectin) have demonstrated some antimicrobial activity, and have been tested as a possible alternative to antibiotics for treating microbial pathogen infections (Pettengill *et al.*, 2012; Lim *et al.*, 2013), they have yielded limited positive results on affecting bacteria (Woerde *et al.*, 2015). Indeed, when first discovered, avermectins were stated as 'lacking significant antibacterial properties' (Burg *et al.*, 1979). Ivermectin functions by targeting the glutamate-gated chloride channels of nematodes, thus rendering the helminth paralysed (Wolstenholme and Rogers, 2005). However, these ion channels are only present in protostome invertebrate phyla (Wolstenholme, 2012), thus bacteria are not affected by this mechanism. Results from the present study also indicate that avermectin anthelmintics largely do not affect the microbiota via perturbation of the helminth community (e.g., through alteration of host immune responses resulting from depletion of infection, see Walk *et al.*, 2010; Rausch *et al.*, 2013).

In order to understand the lack of significant changes in microbiota associated with anthelmintic treatment in the current study, it is first necessary to consider how helminths may induce changes in microbiota composition. Bacteria already present in the host gut, or transmitted by other means (e.g., ingested within food sources), are more likely to colonise the gut during helminth infection, due to immune system suppression (Steenhard *et al.*, 2002) and tissue damage (Murray *et al.*, 1970), and these bacteria may endure after helminth removal. Helminths may also alter microbiota

composition of the host via three-way interactions that also involve the immune system, which are stimulated by helminth infection, and may result in microbial changes in the gut (e.g., Walk *et al.*, 2010; Rausch *et al.*, 2013). While immune responses, such as immunoglobulin antibodies, return to pre-infection levels following anthelmintic treatment (Loukas and Prociv, 2001), this requires the complete eradication of helminth infection, which did not occur in the present study. Thus some immune responses against helminth infection may have remained, maintaining the impact on microbiota composition.

To date, only the current study, and three others (Cooper *et al.*, 2013; Sirois, 2013; Houlden *et al.*, 2015) have investigated the effect of anthelmintic treatment on microbiota. Results from these experiments show a range of effects on the microbial community associated with anthelmintic treatment, including reversion of microbiota composition to one which is more similar to uninfected individuals (Houlden *et al.*, 2015), shifts in *Bacteroidetes*:*Firmicutes* ratios (Sirois, 2013), to very little effect on microbiota composition (Cooper *et al.*, 2013; current Chapter). Interest in this topic is growing due to the potential health and economic consequences of anthelmintic treatment for both humans and livestock. In 2016 a proposal to trial how the anthelmintic albendazole affects microbiota of children was approved (Leung *et al.*, 2016). Indeed, the removal and control of helminths is such a pertinent topic that the effect of non-pharmaceutical anthelmintics on microbiota has also received some interest. For example, chicory roots reportedly have both anthelmintic and antibiotic properties following ingestion, and have been fed to domestic pigs which had been experientially infected with two helminth species (Jensen *et al.*, 2011). While dietary supplementation with chicory roots did successfully decrease the abundance of one helminth species, the other helminth species subsequently showed an increase in abundance, and no significant changes were reported in microbiota composition

(Jensen *et al.*, 2011). It is evident that there are pressing concerns regarding anthelmintic resistance and knock-on effects on microbiota, but at present there have been few studies investigating the effect of anthelmintics/helminth removal on the microbiota, despite potential implications for human and livestock health.

To conclude, diversity of gut microbiota of wild rodents harbouring a natural helminth infection remains mostly stable following anthelmintic treatment, and reduction in helminth infection. This supports previous evidence that the avermectin family of anthelmintics does not have any significant antimicrobial effects (Burg *et al.*, 1979; Woerde *et al.*, 2015). In addition, the results presented here indicate that changes in microbiota composition associated with helminth infection (Cebra, 1999; Maizels *et al.*, 2004; Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Rausch *et al.*, 2013; Kreisinger *et al.*, 2015) may persist after infection load is reduced. There are a number of possible reasons that microbiota does not exhibit significant alterations following anthelmintic treatment, based on the different modes by which helminth infection may elicit affect microbiota in the first place. It is important to acknowledge the small sample sizes of the current study, which may have led to insufficient statistical power to detect significant differences in microbiota composition. In addition, suppression of the immune system by some helminths may allow previously non-abundant bacteria to flourish (e.g., Walk *et al.*, 2010; Rausch *et al.*, 2013), and may persist even after infection has been reduced but not cleared. However, microbiota of faeces did show significant changes in composition following anthelmintic treatment. Given that the eggs of many helminth species are expelled and undergo development within host faeces, and bacteria can affect helminth development (e.g., *H. polygyrus*; and *T. muris*; Hayes *et al.*, 2010, also see Chapter 6), further research into the effect of anthelmintic on faecal microbiota, and subsequent implications for helminth development is a future area of discovery. This study provides evidence that low

doses of anthelmintic do not have any short-term impacts on the microbiota, but the effect of higher doses over prolonged periods, as are sometimes administered to humans and livestock, are unknown.

3.6 Author Acknowledgements

The manuscript resulting from this chapter is authored by:

Emily L. Pascoe, Jakub Kreisinger (Charles University, Prague), Margherita Collini (Fondazione Edmund Mach), Heidi C. Hauffe (Fondazione Edmund Mach), Julian R. Marchesi (Cardiff University and Imperial College) and Sarah E. Perkins (Cardiff University and Fondazione Edmund Mach).

Chapter 4

Does disruption of the gut microbiota with antibiotic affect the helminth population?

"True knowledge exists in knowing that you know nothing"

Socrates

4.1 Abstract

Antibiotics are widely used in humans and animals due to their ability to treat bacterial infections and induce growth in livestock. Despite microbiota sharing the gut niche with macro-organisms, the effect of antibiotic treatment on other components of the gut biome, namely the parasitic helminths, has been given little regard. Here, the effect of antibiotic administration on the helminth community was investigated in a wild, naturally infected rodent system. Helminth prevalence, abundance and fecundity were monitored before and after antibiotic treatment. Following significant changes in taxonomic composition and OTU abundances in gut microbiota induced by antibiotic treatment, helminth prevalence increased. In addition, antibiotic treatment resulted in a 45.4% decrease in mean helminth abundance (p = 0.28). Fecundity (egg shedding and *in utero* egg abundance) of both *Heligmosomoides polygyrus* and *Hymenolepis* spp. significantly increased; egg shedding increased by 362% (p <0.01) and 2,165% respectively, and *in utero* eggs increased by 63.9% in *H. polygyrus* (p = 0.03), but decreased by 14.3% in *Hymenolepis* spp. (p = 0.35). Helminth size also increased for *H. polygyrus* (14.3%; p = 0.70) and significantly for *Hymenolepis* spp. (382.0%; p <0.01). The negative effect of antibiotic on helminth abundance could be driven by the loss of bacteria crucial for helminth survival, either from within the host gut or from helminth-associated microbiota, while those remaining helminths have increased fecundity due to competitive release. The negative impact of antibiotic on helminth abundance has implications for human and animal health, and suggests that antibiotics could be an effective short-term alternative to anthelmintics for reducing helminth infection. However, long-term implications could include increased shedding of helminth eggs into the environment, leading to higher rates of transmission in the population.

4.2 Introduction

Antibiotics have revolutionised human and veterinary medicine: they relatively quickly treat microbial infections by killing pathogenic bacteria or preventing their proliferation (Hauser, 2012), and are exploited for their growth-inducing properties in livestock (Goossens *et al.*, 2005). Antibiotics usually function on a 'broad-spectrum', meaning that many non-target and non-pathogenic bacteria can be affected, often leading to gut dysbiosis; the effects of which can be long-term and observed years after the antibiotic was administered (Kilkkinen *et al.*, 2002; Hawrelak and Myers, 2004; Jernberg *et al.*, 2007; Jakobsson *et al.*, 2010). In addition, over- and improper use of antibiotics have led to an alarming rate of antibiotic resistance in many strains of bacteria (Shlaes, 2010). Concerns related to antibiotic resistance led to an EU ban in 2006 on their use as a growth-promoter in livestock (Anadón, 2006). In spite of this ban, worldwide antibiotic overuse remains widespread in both humans and animals; in Chile alone hundreds of tonnes of antibiotics are used annually in salmon farms (Landers *et al.*, 2012; Cabello *et al.*, 2013; Versporten *et al.*, 2014), while in humans annual worldwide antibiotic consumption is 70 billion standard units (where a unit is equivalent to one pill; (Van Boeckel *et al.*, 2014). In addition, there are minimal restrictions regarding administration of antibiotics to companion animals (Prescott, 2008). Yet we still do not know the full extent of how disrupting gut bacteria by antibiotic affects the whole gut biome, which is comprised of more than just the microbiota.

The gut biome also has a 'macrobiota' component; the parasitic helminths, which may cause malnutrition and reduce fecundity of the host (Shetty, 2010; Sutherland and Scott, 2010), but on the flip-side, can also elicit a protective defence against autoimmune diseases in humans (Bilbo *et al.*, 2011). Parasitic helminths have co-evolved with microbiota within the gut for millennia, and interactions have formed between these two communities (e.g., Glendinning *et al.*, 2014). For

instance, studies have found that helminth infection influences microbiota composition, generally causing an increase in bacterial diversity (Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Rausch *et al.*, 2013), with effects sometimes observable up- or down-stream from the site of infection (Kreisinger *et al.*, 2015). Likewise, the consortia of bacteria present in the host gut can affect the susceptibility of an individual to helminth infection (Martínez-Gómez *et al.*, 2009; Hayes *et al.*, 2010; Coêlho *et al.*, 2013). Since bacteria can influence helminth infection, depletion or disruption of the microbiota is also likely to affect the helminth community.

There is already some evidence that antibiotics affect the helminth community. Antibiotics were tested as a possible method of treating helminth infection more than half a century ago. Results were promising; antibiotics such as chlortetracycline hydrochloride, oxytetracycline and bacitracin reduced pinworm abundances in mice and humans by up to 80%, while in some individuals the infection was entirely removed (Wells, 1951, 1952a, 1952b), and the gut remained uninfected for up to 72 hours after treatment (Chan, 1952). Cestodes were also successfully removed in humans treated with paromomycin (Salem and el-Allaf, 1969). Even substances with weak antibacterial effects, such as gentian violet, reduced helminth abundances by around 50% (Brown, 1952; Wells, 1951). In addition, helminths that remained following antibiotic administration were smaller in size, and fecundity and virulence were reduced (Wells, 1951; Brown, 1952; Chan, 1952; Wells, 1952a, 1952b; Salem and el-Allaf, 1969; Hoerauf *et al.*, 1999; Saint André *et al.*, 2002). However, antibiotics did not consistently have a negative effect on helminth abundance; administration of neomycin, dihydrostreptomycin and chloramphenicol resulted in increased helminth abundance (Wells, 1952a). The majority of these studies were performed before the advent of metataxonomics, and mainly used a laboratory model system infected with a single helminth

species, thus were not able to assess if and how the microbiota changed following antibiotic treatment, nor how a replete microbiota and helminth community would respond to antibiotic.

Although initial studies simply observed the effect of antibiotic on the helminth community, more recent work has attempted to tease apart the mechanisms by which removal of bacteria may impact the macrobiota. For example, studies have indicated that parasite establishment is less successful following antibiotic treatment, as helminths may rely on a 'service' provided by bacteria (e.g., carbohydrate digestion; Biswal *et al.*, 2016, or to initiate egg hatching; Hayes *et al.*, 2010), which is disrupted by the effect of antibiotic on the respective bacteria. Conversely, antibiotics may influence the abundance of helminths or other endoparasites through changes in host immune responses associated with the removal of microbiota (Mathis *et al.*, 2005), or by killing the symbiotic bacteria crucial for helminth survival (e.g., *Wolbachia* in filarial nematodes; Saint André *et al.*, 2002).

It is apparent that antibiotics can affect some helminth species in laboratory animals, possibly due to a cascade effect of disrupting the gut microbiota. However, as yet, antibiotic-helminth interactions have not been investigated in a wild system harbouring a full, interacting consortia of microbiota and macrobiota (Lello *et al.*, 2004; Telfer *et al.*, 2010; Glendinning *et al.*, 2014). The aim of the current study is to establish if microbiota perturbation by antibiotic treatment of a host affects parasitic helminth abundance, fecundity or size in a natural, replete system. A wild rodent, the yellow-necked mouse (*Apodemus flavicollis*) was used as a model system. A cocktail of antibiotics which has previously been reported to deplete the cultivable microbiota of laboratory mice (Reikvam *et al.*, 2011) was administered to *A. flavicollis* individuals. Helminth eggs per gram of faeces were continuously monitored pre- and post-treatment of each individual.

Furthermore, a subset of individuals from each treatment population were euthanised pre- and post-treatment, from which helminths were analysed and microbiota was characterised to assess any changes in diversity, composition and OTU abundances following treatment. Preliminary analyses of helminth eggs per gram of faeces indicated that egg shedding increased post-antibiotic treatment, and in order to establish if this increased egg shedding resulted from an increase in females or an increase in the fecundity of females already present in the population resulting from antibiotic treatment, the female sex ratio (for sexually dimorphic species) and *in utero* egg count were also monitored in helminths from pre- and post-treatment individuals. Helminth size was also measured following previous reports of a decrease in size after antibiotic treatment (Wells, 1951).

4.3 Materials and methods

4.3.1 Study area and small rodent sampling

Live-trapping of *Apodemus flavicollis* was conducted using Ugglan multi-capture traps (Ugglan Type 2; Grahnab, Sweden) arranged in four grids of 64 traps each (8×8), with a 10 m inter-trap interval. Two grids were established at the locality of Cavedine (45°59'21.2"N, 10°57'59.6"E and 45°58'30.8"N, 10°57'22.0"E) and two at Pietramurata (46°01'01.4"N, 10°55'22.8"E and 46°00'47.7"N, 10°55'40.7"E) in the Province of Trento (Italy). Each grid occupied woodland with a similar vegetation composition and structure, and was situated at least 500 m from neighbouring grids to minimise inter-grid movement of animals. Trapping grids at each locality were randomly assigned to either antibiotic or sham control treatment. Traps were baited with sunflower seeds and potato for two nights on a consecutive biweekly basis, at each locality, from mid-May to August 2014. Following this pre-treatment monitoring of microbiota and macrobiota, trapping

was conducted at both localities intensively for four nights on a weekly basis during the treatment (August) and post-treatment monitoring periods (end of August to September). Throughout the course of trapping, a total of 147 individuals were captured, 57 from antibiotic assigned grids and 90 from control assigned grids. However some of these individuals were excluded from analyses as they were not re-captured following treatment; of these 147 mice 64.6% were captured on more than one occasion; 61.4% in antibiotic assigned grids and 64.8% in control assigned grids. Animal trapping and handling procedures were authorised by the Comitato Faunistico Provinciale della Provincia di Trento, prot. n. 595 issued on 04 May 2011.

Upon initial capture, each mouse was tagged with a subcutaneous passive integrated transponder (Trovan ID 100; Ghislandi and Ghislandi, Italy), so that each individual could be identified at subsequent captures. Host body mass, sex and breeding status were recorded. Mice were regarded as juveniles if the pelage indicated that the post-juvenile moult had not yet occurred (Gurnell *et al.*, 1990), whilst adults were categorised according to breeding condition (descended testes for males and perforated vagina or pregnant for females; Gurnell *et al.*, 1990); individuals with adult pelage which were not in breeding condition were classified as sub-adults. Each week, faeces collected at first capture of an individual were collected for faecal egg count (FEC) analyses, using a standard McMaster technique with saturated NaCl flotation solution, and helminth eggs per gram of faeces (EPG) was calculated (after Dunn and Keymer, 1986). When an individual was captured more than once during a trapping week, subsequent faecal samples were collected for microbiota analyses, and were transported to the laboratory at 4°C, whereupon they were immediately frozen at -80°C until DNA extraction (see '*4.3.6 16S rRNA gene sequencing'* below). A total of 26 mice (6 untreated and 7 treated from the antibiotic group, and 6 untreated and 7

treated from the control group) were randomly selected throughout the course of the experiment, and euthanised by overdose of isoflurane, followed by cervical dislocation.

4.3.2 Microbiota manipulation

During an 18-day period in August 2014 all adult and sub-adult mice captured at each grid were administered up to three doses of the respective treatment, with a minimum of seven days between each dose. The antibiotic treatment consisted of a solution of 5 mg/ml vancomycin, 10 mg/ml neomycin, 10 mg/ml metronidazol, 10 mg/ml ampicillin and 0.1 mg/ml amphotericin B (Sigma-Aldrich, USA), dissolved in sterile PBS solution (after Reikvam *et al.*, 2011). The antibiotic solution was vigorously vortexed for 10 minutes each day before use. The sham control consisted of a dose of ultra-pure water. Each treatment was administered using a curved gavage needle (18 G × 50 mm) at a dose of 2 ml/Kg (adapted from Reikvam *et al.*, 2011). Due to the vagaries of trapping wild animals, not every individual was captured three times/with a sufficient time interval between doses throughout the treatment period to receive the intended three doses of treatment: a total of 25 individuals were treated with antibiotic (one dose n=8, two doses n=9, three doses n=8), while due to a difference in population densities, 42 individuals were treated with the control sham gavage (one dose n=30, two doses n=11, three doses n=1).

4.3.3 Analyses of gut samples

The 26 euthanised *A. flavicollis* were dissected under sterile conditions following methods in Kreisinger *et al.*, (2015). Briefly, the gut was washed in sterile Tris-buffered saline (TBS; Tris-NaCl; 50 mM Tris, 200 mM NaCl, pH8) and separated into four functional sections (stomach, small intestine, caecum, and colon). The luminal contents and membrane of each section was diluted with TBS and scanned for helminths at 10× magnification (Leica© MS5 microscope with

a Leica© CLS100 light attachment). Helminths were collected according to species, gut section and mouse individual in 70% ethanol for future size and fecundity analyses (see '*4.3.4 Helminth size and fecundity measurements*'). After thoroughly scraping the gut membrane with tweezers under TBS to dislodge bacteria, the membrane and the TBS containing bacteria were collected with the rest of the luminal contents in a centrifugation tube. A bacterial pellet was obtained by centrifugation (950 G for 10 minutes at 4°C, resulting supernatant 9,000 G for 15 minutes at 4°C. The membrane did not form part of the pellet during the second centrifugation and was discarded). The bacterial pellet was immediately stored at -80°C for future bacterial DNA analysis (see '*4.3.6 16S rRNA gene sequencing*' below).

4.3.4 Helminth size and fecundity measurements

Helminths were removed from storage in 70% ethanol and submerged in sterile water for one hour to 'relax' brittle helminths; a condition associated with ethanol storage. Individual helminths were transferred onto a slide and fixed/cleared using 70% ethanol and 100% glycerol in a volume ratio of 1:1 (*Heligmosomoides polygyrus*, *Hymenolepis* spp., *Trichuris muris*; adapted from Berland, 1984) or 1:1 of 70% ethanol and lactophenol (*Aspicularis tetraptera, Syphacia frederici* and *Trichuris muris*). Due to their size or transparency, *Mastophorus muris* and *Corrigia vitta* could not be/did not require fixing/clearing. Each helminth was photographed at 10× magnification using a Leica© DFC420C camera attached to a Leica© MZ75 microscope. Leica© software was used to provide a fine scale for each image, and from these photographs the length and width (at three random points along the length) of each helminth was measured using ImageJ software from which to calculate helminth area. At this stage it was also possible to identify *Hymenolepis* from two species; *H. diminuta* and *H. straminea.* Female helminths from *H. polygyrus*, *S. frederici* and *A. tetraptera* were photographed using a Leica© DMLB microscope at 50× magnification to

perform an *in utero* egg count as a proxy for fecundity. An *in utero* egg count was performed on *T. muris*, and the three posterior proglottids from each *Hymenolepis* (including pieces of *Hymenolepis* from which the scolex had detached), by macerating the helminth/proglottids, in sterile water and observing at 100× magnification. For *Hymenolepis* spp. the mean egg count of the three proglottids was multiplied by the number of mature proglottids from all *Hymenolepis* within a mouse, and divided by the number of scolices found, to give an *in utero* egg count/helminth accounting for proglottids that had detached from scolices. Preliminary analyses found that the number of eggs did not differ substantially between mature proglottids of the same helminth.

4.3.5 Statistical analyses of helminth EPG, abundance, fecundity, percentage of females and size

A total of 1,179 helminths were collected from 26 euthanised mice, of which 1,001 were in a condition which allowed further analysis of size and *in utero* egg counts (178 were lost/damaged after quantification during host dissection). A total of 134 FEC measurements, which included at least one pre- and one post-treatment sample from any given individual (79 FECs from 12 individuals in the antibiotic group and 55 FECs from 14 individuals in the control group) were used for statistical analyses of EPG. Generalised linear mixed models (GLMM) were used to test for significant differences in helminth abundance (total number of helminths present, including zero values of uninfected hosts, as defined by Bush *et al.*, 1997) and helminth EPG (here defined as the total number of helminth eggs present in faeces, including zero values of uninfected hosts) associated with antibiotic treatment. In addition, GLMMs were run to test for significant differences in fecundity (*in utero* egg counts), the percentage of females (in sexually dimorphic helminth species) and helminth size of both *H. polygyrus* and *Hymenolepis*. Due to a lack of

statistical power (abundance <5, or present in only one individual), the other species could not be analysed separately for any of these parameters, but were included in analyses of total helminth prevalence, abundance and EPG analyses. Preliminary analyses indicated that data had insufficient power to include treatment interacting with treatment period (antibiotic and control data pooled) as an explanatory variable, thus antibiotic and control data were analysed in separate GLMMs; firstly a GLMM was used to test that there were no significant differences in helminth abundance, EPG, fecundity, female percentage and size in pre-treatment individuals between the antibiotic and control group to ensure changes in post-treatment individuals were comparable. Once this assumption was confirmed, for all GLMMs host sex, host breeding status, host body mass and treatment period (pre- or post-treatment) were explanatory variables. In addition, the model included the following two-way interaction terms as explanatory variables: all possible two-way interactions between host sex, host breeding status and host body mass. The identity code of the individual, geographical location (Cavedine or Pietramurata) and sampling month were all modelled as a nested random intercept for each model. For each GLMM, a process of multi-model inference was used to compare all possible models using the R package 'MuMIn' (Bartoń). The most parsimonious model was selected using a threshold of ΔAICc <2 (Burnham and Anderson, 2003). Statistical analysis used the package 'glmmADMB', version 8.3.3 (Fournier *et al.*, 2012; Skaug *et al.*, 2016).

4.3.6 16S rRNA gene sequencing

A total of 53 frozen faecal samples, which included at least one pre- and one post-treatment sample from any given individual, were sequenced for microbiota analyses; 34 samples from 14 individuals from the antibiotic group, and 19 samples from 8 individuals from the control group. Small intestine, caecum and colon samples from 26 individuals were sequenced. The QIAmp

DNA Stool Mini kit (Qiagen, Valencia, CA, USA) was used for total genomic DNA extraction from each luminal bacteria sample (small intestine, caecum and colon, the microbiota of the stomach was not analysed) and from faeces. In addition to the methods provided by the manufacturer for pathogen detection, a 2 minute homogenisation step at 30 Hz was performed to enhance bacterial cell lysis, using a Mixer Mill MM200 (Retsch GmbH, Haan, Germany) with 5 mm stainless steel beads (Qiagen, Valencia, CA, USA). Purity and quality of the recovered DNA were determined using a QIAxcel capillary electrophoresis system (Qiagen, Valencia, CA, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified using the 341F and 805R primers (see Appendix A.2, Figure A.2.1 for details on primer sequences, including degenerate nucleotides), and sequenced using a 2×300 bp kit on the Illumina MiSeq system (Illumina, San Diego, CA, USA). The PCR reactions were carried out in a total volume of 25 µl containing 0.4 µM of each primer, 0.4 mM of dNTP (Promega, Madison, WI, USA), 1× FastStart reaction buffer (Roche Diagnostics GmbH, Mannheim, Germany), 1 mM of $MgCl_2$, 1.25 unit of FastStart HiFi Polymerase (Roche Diagnostics GmbH, Mannheim, Germany), and 12.5 ng of genomic DNA for each sample amplification. Thermal cycling was performed on a GeneAmp™ PCR System 9700 instrument (Thermo Fisher Scientific, Waltham, MA, USA) as follows: initial denaturation at 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 55°C for 45 seconds, 72°C for 1 minute 15 seconds, and a final extension at 72°C for 8 minutes. Negative controls for extraction and PCR reactions were included, and genomic DNA from Microbial Mock Community B (Staggered, Low Concentration), v5.2L (BEI Resources, Manassas, VA, USA) was included to assess the effect of data processing on observed community content. Dual indices and Illumina sequencing adapters were attached using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). The final library was cleaned, quantified, normalised and pooled in an equimolar way before sequencing on the Illumina MiSeq system (Illumina, San Diego, CA, USA) at the

University of Trento, Trento, Italy. Sequencing was carried out following the manufacturer's recommendations.

4.3.7 Bioinformatic processing of 16S data

Sequences were merged, trimmed and filtered using MICCA software (version 1.5.0, Albanese *et al.*, 2015). Overlapping regions of the forward and reverse read sequences that differed by more than eight nucleotides, or did not contain both the forward and reverse PCR primer sequences were discarded. The resulting, merged 16S fragments were discarded if they had an average expected error (AvgEE) greater than 0.1. Operational taxonomic units (OTUs) were assigned using a *de novo*, greedy strategy using a cut-off of 97% similarity based on the VSEARCH clustering algorithm implemented in MICCA (Rognes *et al.*, 2016). Chimeric sequences were discarded. Resulting representatives of each OTU were classified using the Ribosomal Database Project classifier (RDP classifier, version 2.12; Michigan State University [http://rdp.cme.msu.edu/]). Samples that had final read counts of less than 10,000 merged and quality-filtered reads were discarded. The resulting OTUs were analysed at the phylum and class level using Phyloseq version 1.16.2 (McMurdie and Holmes, 2013).

4.3.8 Statistical analyses of microbiota - diversity

GLMMs were used to assess whether there was an association between microbiota alpha diversity (inverse Simpson index) and antibiotic treatment. Preliminary analyses indicated that data had insufficient power to include treatment interacting with treatment period (antibiotic and control data pooled) as an explanatory variable, thus antibiotic and control data were analysed in separate GLMMs; firstly a GLMM was used to test that there were no significant differences in microbiota alpha diversity in pre-treatment individuals between the antibiotic and control group to ensure

changes in post-treatment individuals were comparable. Once this assumption was confirmed separate GLMMs were run with alpha diversity of either the small intestine, caecum, colon, whole gut (small intestine, caecum and colon combined) or faeces as the response variable. Host sex, breeding status and treatment period (pre- or post-treatment) were explanatory variables. The identity code of the individual, geographical location (Cavedine or Pietramurata) and sampling month were each modelled as a nested random intercept for each model. A process of multi-model inference was used to compare all possible models using the R package 'MuMIn' (Bartoń), and the most parsimonious model was selected using a threshold of ΔAICc <2 (Burnham and Anderson, 2003).

4.3.9 Statistical analyses of microbiota - composition

A distance-based redundancy analysis (db-RDA; capscale function in R package *vegan*) was used to test for differences in microbiota composition associated with antibiotic treatment, in the small intestine, caecum, colon, whole gut (small intestine, caecum and colon combined) or faeces. Ecological distances between microbiota communities from pre-treatment and post-treatment individuals (for both antibiotic and control) were assessed using Bray–Curtis dissimilarities (i.e., compositional dissimilarity index that accounts for proportional differences of OTUs among samples) and weighted UniFrac dissimilarity matrices (which accounts both for proportional differences of OTUs and their phylogenetic relatedness; Lozupone and Knight, 2005). OTU tables were scaled before calculation of dissimilarity matrices to achieve an even sequencing depth, corresponding to the minimal number of reads per sample in gut sections that were included in a given analysis. Significance was assessed using permutation-based marginal tests.

4.3.10 Statistical analyses of microbiota - OTU abundances

To determine how OTU abundances varied following antibiotic treatment, OTUs with a differential abundance (i.e., number of reads corrected for sequencing depth) between pre- and post-treatment individuals in the whole gut, each gut section and in faeces were first identified, using an approach based on generalised linear models with negative binomial errors implemented in the DESeq2 package (Anders and Huber, 2010). These analyses were run using the default pipeline set-up in DESeq2, and significance values (p >0.05) were derived using likelihood-ratio tests.

4.4 Results

4.4.1 The effect of antibiotic treatment on helminth prevalence and abundance

Antibiotic treatment was associated with a 50.0% and 14.3% increase in prevalence of *H. polygyrus* and *Hymenolepis* spp., respectively in post- compared to pre-treatment individuals (Figure 4.1). Similarly, in the control group prevalence increased for *H. polygyrus* (20.0%) and *Hymenolepis* spp. (50.0%) in post- compared to pre-treatment individuals. Antibiotic treatment generally had a negative impact on helminth abundance, mean abundance decreased by 45.4% between pre- and post-treatment (from 55.5 ± 23.6 helminths to 30.3 ± 7.4), however this decrease was not significant (d.f. = 9, Z = -1.07, p = 0.28; Figure 4.1 and 4.2), and in the control group total helminth abundance also decreased, by 66.6% (75.3 ± 43.9 to 25.1 ± 10.7; d.f. = 10, Z = -0.64, p = 0.52). *Heligmosomoides polygyrus* showed a 14.5% increase in abundance post-antibiotic treatment (from 23.8 ± 17.5 to 27.3 ± 6.5; d.f. = 9, Z = 0.18, p = 0.85), but in the control group *H. polygyrus* abundance decreased post-treatment by 37.6% (from 11.7 ± 5.0 to 7.3 ± 1.6; d.f. = 8, Z = -0.84, p = 0.40), however neither of these changes in abundance were significant

(Figure 4.1 and 4.2). *Hymenolepis* spp. decreased by 86.8% in individuals treated with antibiotic (from 20.5 ± 16.9 to 2.7 ± 1.5), and conversely increased by 75.6% in the control group (from 10.2 ± 4.8 to 17.9 ± 11.2; Figure 4.1 and 4.2), however, treatment was not significantly associated with these changes in either the antibiotic (d.f. = 9, Z = -1.05, p = 0.30) or control (d.f. = 9, Z = 0.70, p = 0.49) group.

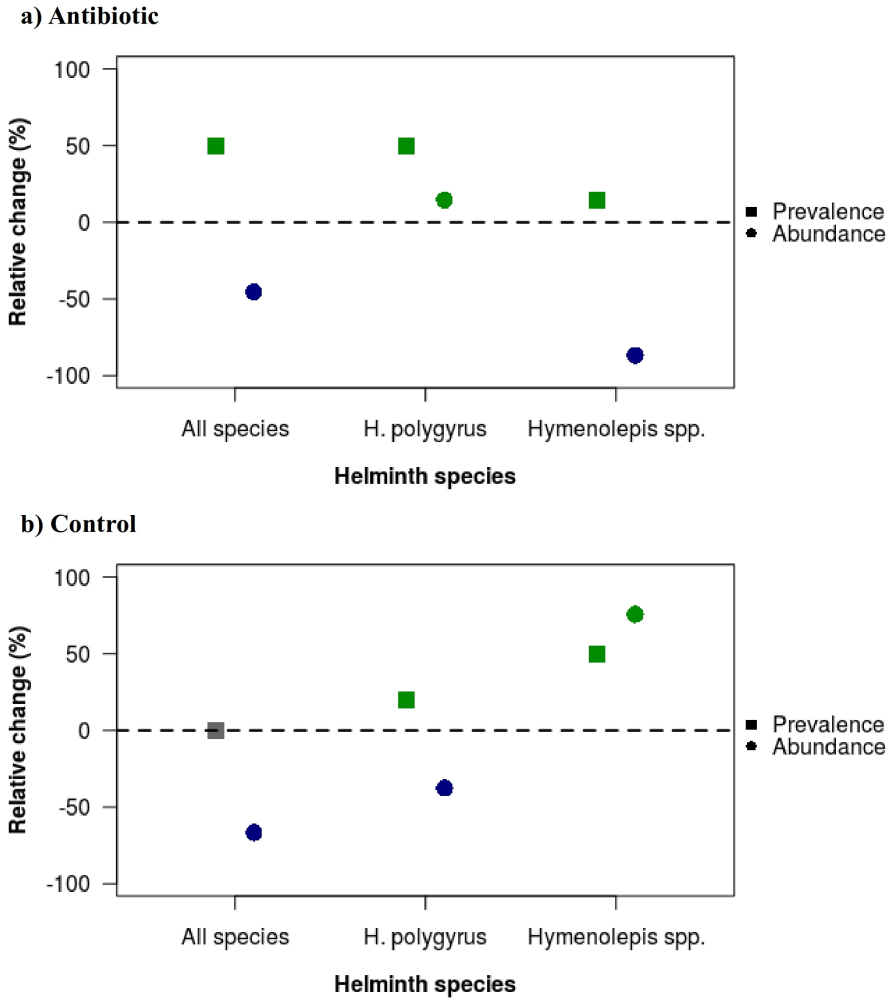

Figure 4.1: Relative changes (%) in helminth prevalence and abundance between pre- and post-treatment individuals in an a) antibiotic and b) control group for all helminth species, *Heligmosomoides polygyrus* and *Hymenolepis* spp. Prevalence and abundance of other identified species were insufficient to perform statistical analyses. Blue data points indicate where there was a relative decrease, green indicates a relative increase and grey indicates where no change was observed between pre- and post-treatment individuals.

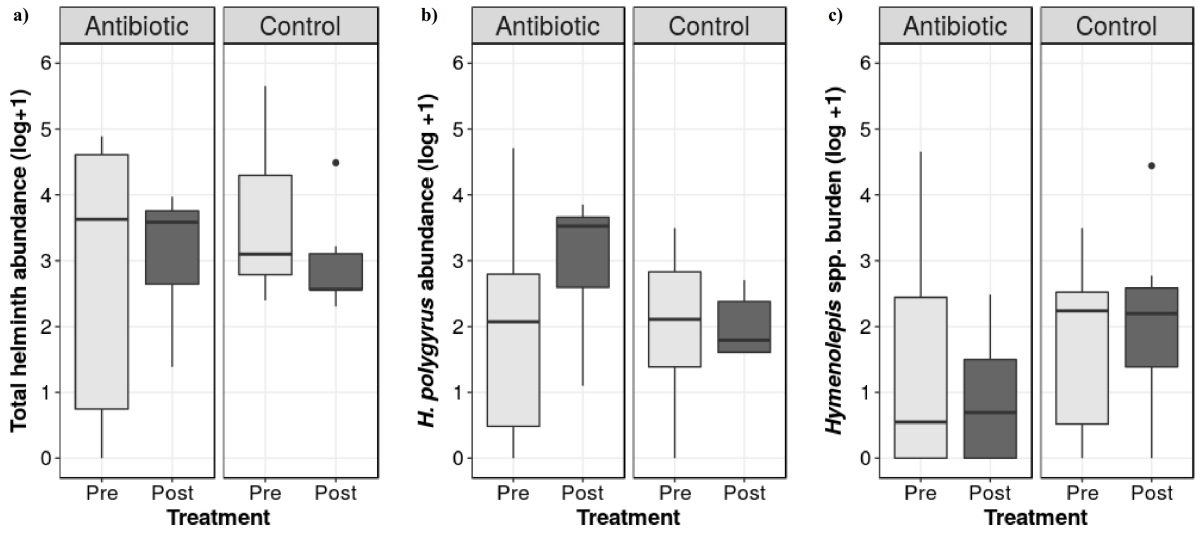

Figure 4.2: Abundance of helminths, including zeros from uninfected individuals (log +1) of a) all species, b) *Heligmosomoides polygyrus*, c) *Hymenolepis* spp. in pre- and post-treatment individuals, in both an antibiotic treatment and control group. Boxes demonstrate the upper and lower quartiles, with median abundance indicated. Bars represent the minimum and maximum range of abundance.

4.4.2 The effect of antibiotic treatment on helminth EPG

Antibiotic treatment was linked to consistent and substantial increases in helminth EPG; mean EPG increased by 790.1% from pre- to post-treatment (from 154.5 ± 46.7 to 1,375.4 ± 289.6 EPG) in the antibiotic group (d.f. = 75, $Z = 2.58$, $p = 0.01$; Figure 4.3 and 4.4). Helminth EPG also increased post-treatment in the control group, by 49.0%, but not significantly so (from 546.5 ± 223.2 to 814.3 ± 246.8; d.f. = 50, $Z = 0.75$, $p = 0.45$). There was a positive effect of antibiotic treatment on *H. polygyrus* EPG, which increased significantly (d.f. = 75, $Z = 2.66$, $p < 0.01$), by 362.4% in post- compared to pre-treatment individuals (from 117 ± 38.3 to 545.0 ± 120.1 EPG; Figure 4.3 and 4.4), but in the control group there was a 95.7% decrease in *H. polygyrus* EPG post- compared to pre-treatment (from 207.6 ± 98.3 to 172.0 ± 68.4), which was not significant (d.f. = 51, $Z = -0.55$, $p = 0.58$). EPG of *Hymenolepis* also significantly increased in the antibiotic

group (d.f. = 74, Z = 2.24, p = 0.03), by 2,164.7% in post- compared to pre-treatment (36.7 ± 31.7 to 830.4 ± 276.7 EPG; Figure 4.3 and 4.4). In the control group *Hymenolepis* EPG was also higher in post- compared to pre-treatment individuals, but only by 95.7% (317.8 ± 212.6 to 622.1 ± 252.2; d.f. = 51, Z = 0.58, p = 0.56).

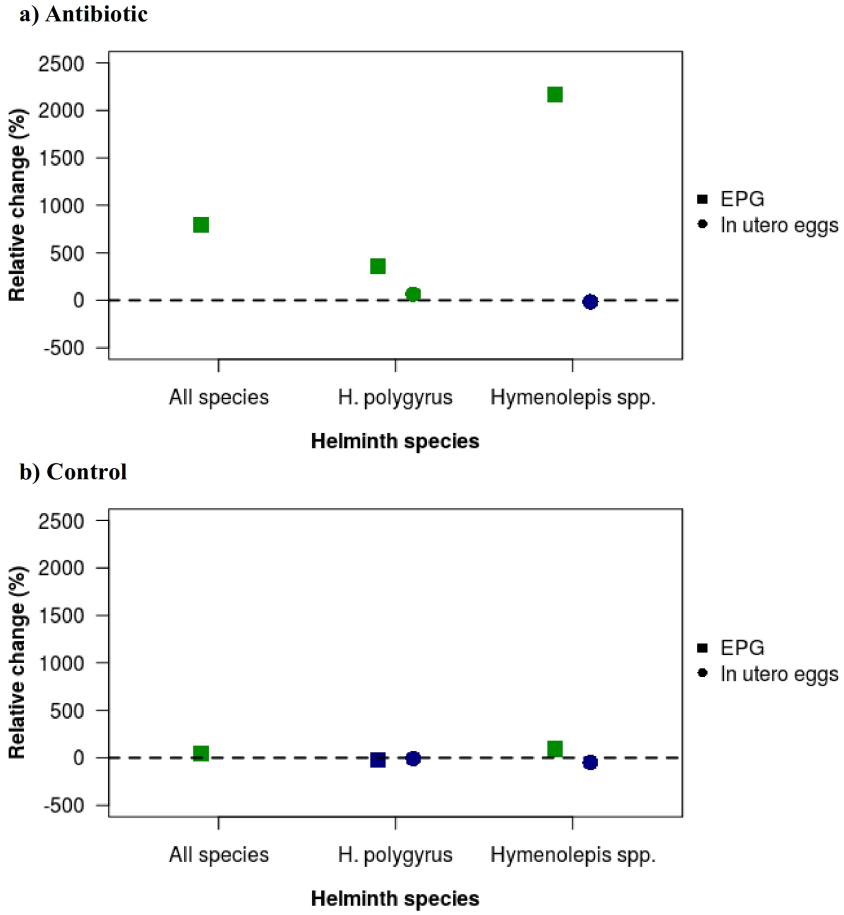

Figure 4.3: Relative changes (%) in eggs per gram (EPG) of faeces and *in utero* egg abundance between pre- and post-treatment individuals in an a) antibiotic and b) control group for all helminth species, *Heligmosomoides polygyrus* and *Hymenolepis* spp. Prevalence and abundance other identified species were insufficient to perform statistical analyses. Blue data points indicate where there was a relative decrease, green indicates a relative increase and grey indicates where no change was observed between pre- and post-treatment individuals.

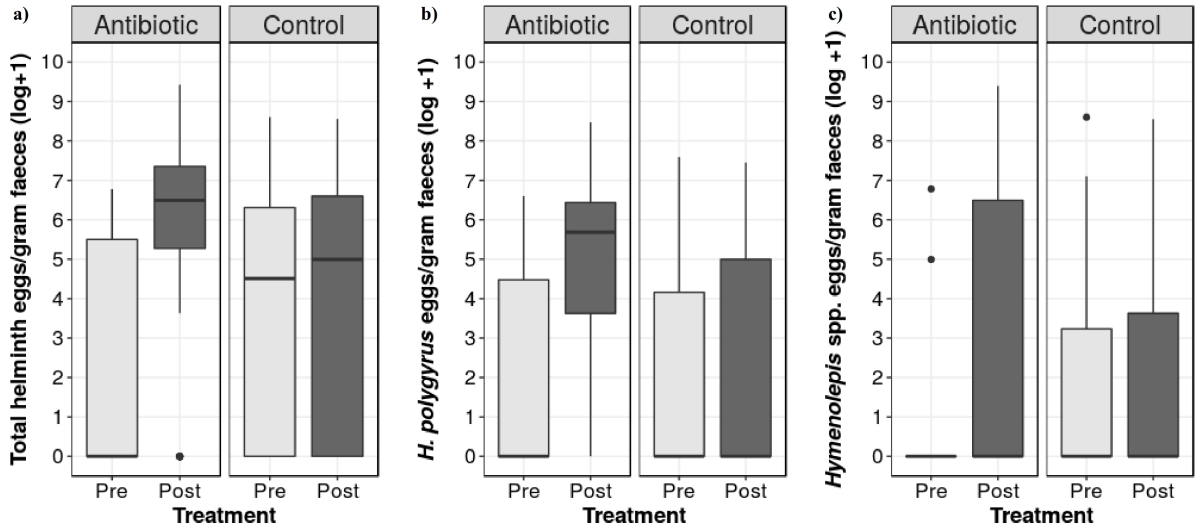

Figure 4.4: Helminth eggs per gram (EPG) of faeces (log +1) of a) all species, b) *Heligmosomoides polygyrus*, c) *Hymenolepis* spp. in pre- and post-treatment individuals, in both an antibiotic treatment and control group. Boxes demonstrate the upper and lower quartiles, with median EPG indicated. Bars represent the minimum and maximum range of EPG.

4.4.3 The effect of antibiotic treatment on helminth fecundity

The effect of antibiotic treatment on helminth fecundity (*in utero* egg count) was species specific, but in the control group post-treatment individuals consistently had lower fecundity than pre-treatment individuals (Figure 4.3). In the antibiotic group, *H. polygyrus* fecundity was 63.9% higher; from 23.6 ± 3.7 *in utero* eggs pre-treatment compared to 37.9 ± 9.2 post-treatment (d.f. = 93, Z = 1.00, p = 0.32; Figure 4.3 and 4.5), while in the control group *H. polygyrus* fecundity decreased by 0.8% (from 16.5 ± 3.0 to 32.9 ± 3.6; d.f. = 48, Z = -0.43, p = 0.67; Figure 4.3 and 4.5). *Hymenolepis* showed a 73.8% decrease in fecundity post- compared to pre- antibioitc treatment, from 220.5 ± 25.7 *in utero* eggs to 57.7 ± 13.1 (d.f. = 40, Z = -0.65, p = 0.52), and in the control group there was a 240.6% decrease in *Hymenolepis* fecundity post- compared to pre-treatment (data insufficient for GLMM).

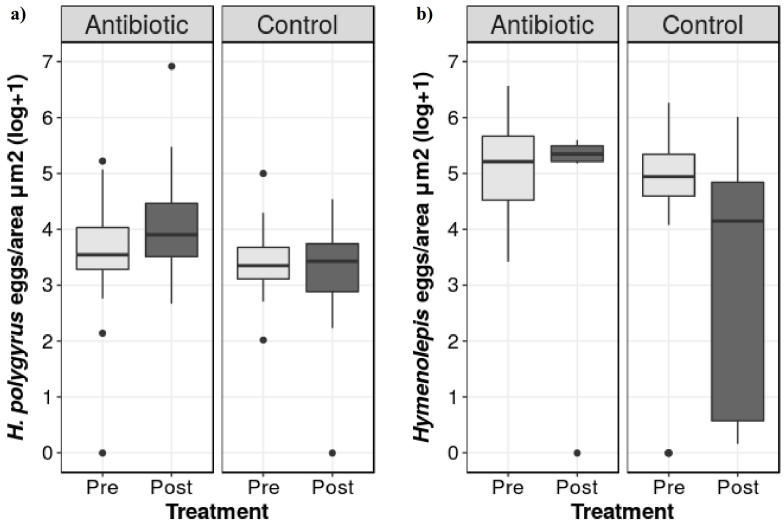

Figure 4.5: *In utero* egg count/area µm^2 (log +1) as a proxy for helminth fecundity of a) *Heligmosomoides polygyrus*, b) *Hymenolepis* spp. in pre and post individuals in both an antibiotic and control treatment population.

4.4.4 The effect of antibiotic treatment on percentage of female helminths

Antibiotic treatment had no significant effect on the percentage of helminths that were female; there were 1.7% more female *H. polygyrus* in post- compared to pre-treatment individuals (from 50.9% to 51.7%; d.f. = 7, Z = 0.63, p = 0.53). Instead, the percentage of female *H. polygyrus* decreased by 15.4% in post- compared to pre-treatment individuals in the control group (from 64.6% to 54.7%; d.f. = 9, Z = 0.38, p = 0.70).

4.4.5 The effect of antibiotic treatment on helminth size

Antibiotic treatment was associated with an increase in helminth size, in comparison to the control group in which all helminths were smaller post-treatment. The size of *H. polygyrus* increased by 14.2% post- compared to pre- antibiotic treatment, from 0.62 µm^2 ± 0.04 to 0.71 µm^2 ± 0.06 (d.f. = 209, Z = 0.39, p = 0.70), and were 5.7% smaller post- compared to pre- treatment in the control

group, from 0.85 μm^2 ± 0.08 to 0.80 μm^2 ± 0.06 (d.f. = 105, Z = -0.17, p = 0.86). *Hymenolepis* were 229.5% bigger in post- compared to pre- antibiotic treatment individuals, from 9.30 μm^2 ± 2.69 to 57.68 ± 13.1 μm^2 (d.f. = 44, Z = 4.06, p <0.01), and instead *Hymenolepis* were 24.1% smaller post- compared to pre-treatment in the control group (data insufficient for GLMM).

4.4.6 The effect of antibiotic on gut and faecal microbiota diversity

The sequences from one faecal and one small intestine sample were discarded as they did not meet the quality filtering criteria. The filtered dataset consisted of 2,896,364 high-quality reads from 124 samples (mean ± standard error = 23,358 ± 32,124, range = 10,073-49,083), within which 14 phyla were identified. Alpha diversity of small intestine microbiota increased post- compared to pre-treatment for the antibiotic group (d.f. = 7, Z = 1.89, p = 0.06), and significantly so for the control group (d.f. = 10, Z = 2.71, p <0.01; Figure 4.6). Antibiotic treatment reduced the alpha diversity of caecum microbiota (d.f. = 7, Z = -0.98, p = 0.33), but in the control group caecum alpha diversity increased post- compared to pre-treatment (d.f. = 7, Z = 0.77, p = 0.44; Figure 4.6). In the colon, alpha diversity decreased in post-treatment individuals for both the antibiotic (d.f. = 8, Z = -1.14, p = 0.25) and the control group (d.f. = 10, Z = -0.06, p = 0.96; Figure 4.6). Alpha diversity of faeces was lower in post-treatment compared to pre-treatment individuals for both the antibiotic (d.f. = 30, Z = -1.60, p = 0.11), but increased between pre- and post-treatment individuals in the control group (d.f. = 16, Z = 0.22, p = 0.82; Figure 4.6). For faeces, alpha diversity decreased following treatment in both the antibiotic (d.f. = 29, Z = -1.60, p = 0.11) and in post- control treatment individuals (d.f. = 14, Z = 0.22, p = 0.82).

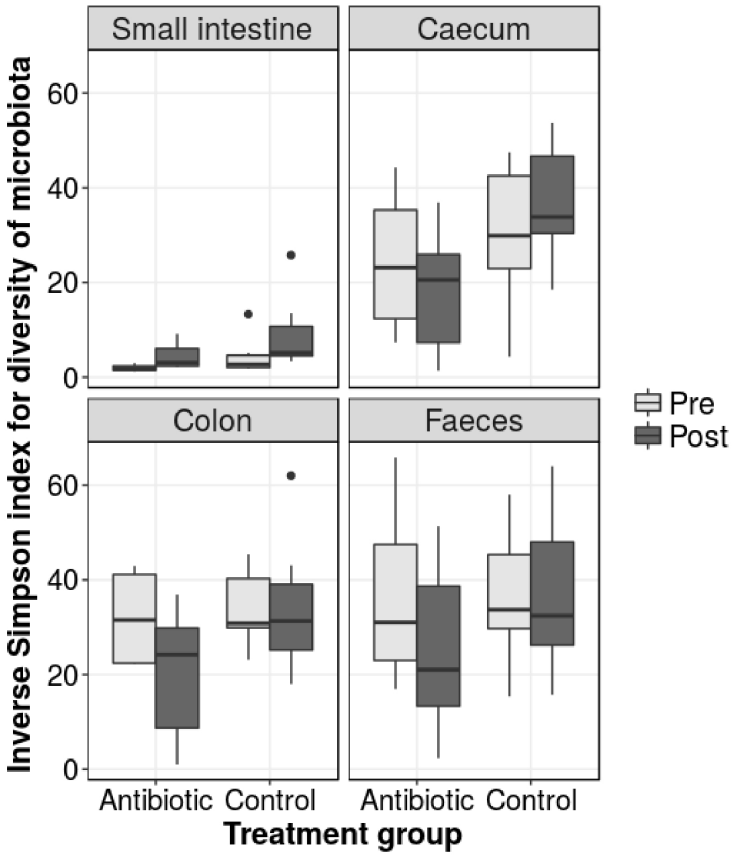

Figure 4.6: Inverse Simpsons diversity index for microbiota in different gut sections and faeces of pre- and post-treatment mice in an antibiotic or control group. Boxes demonstrate the upper and lower quartiles of alpha diversity, with median alpha diversity indicated. Bars represent the minimum and maximum range of alpha diversity.

4.4.7 The effect of antibiotic on gut and faecal microbiota composition

In brief, the majority of all reads from gut and faecal microbiota were from the phylum *Firmicutes* (39.2%), followed by *Bacteroidetes* (38.3%) and *Proteobacteria* (15.5%; Figure 4.7). Of note, 22.9% of reads from small intestine samples were of the phylum *Tenericutes*. At the class level, the majority of reads were *Bacteroidia* (37.9%), *Clostridia* (31.6%) and *Gammaproteobacteria* (11.1%), plus in the small intestine 29.4% of reads were *Bacilli* and 22.7% were *Mollicutes* (Figure 4.7). Antibiotic treatment was associated with significant changes in

taxonomical composition of microbiota for all gut sections, with the exception of the small intestine (Bray-Curtis: d.f. = 19, F = 1.18, p =0.20; weighted UniFrac: d.f. = 19, F = 0.89, p = 0.57; Figure 4.7, 4.8, 4.9 and 4.10). Significant differences in taxonomic composition of microbiota in post- compared to pre-treatment individuals were observed in whole gut (Bray-Curtis: d.f. = 67, F = 2.37, p <0.01; weighted UniFrac: d.f. = 67, F = 3.23, p <0.01; Figure 4.8), caecum (Bray-Curtis: d.f. = 20, F = 1.46, p = 0.01; weighted UniFrac: d.f. = 20, F = 2.7, p = 0.02; Figure 4.9), and colon microbiota (Bray-Curtis: d.f. = 20, F = 1.29, p = 0.02; weighted UniFrac: d.f. = 20, F = 2.14, p = 0.03; Figure 4.9). Faeces only partially followed this pattern; microbiota showed a significant change in taxonomic composition post-antibiotic treatment, but only according to Bray-Curtis dissimilarities (Bray-Curtis: d.f. = 49, F = 1.88, p <0.01; weighted UniFrac: d.f. = 49, F = 1.3, p = 0.17; Figure 4.10).

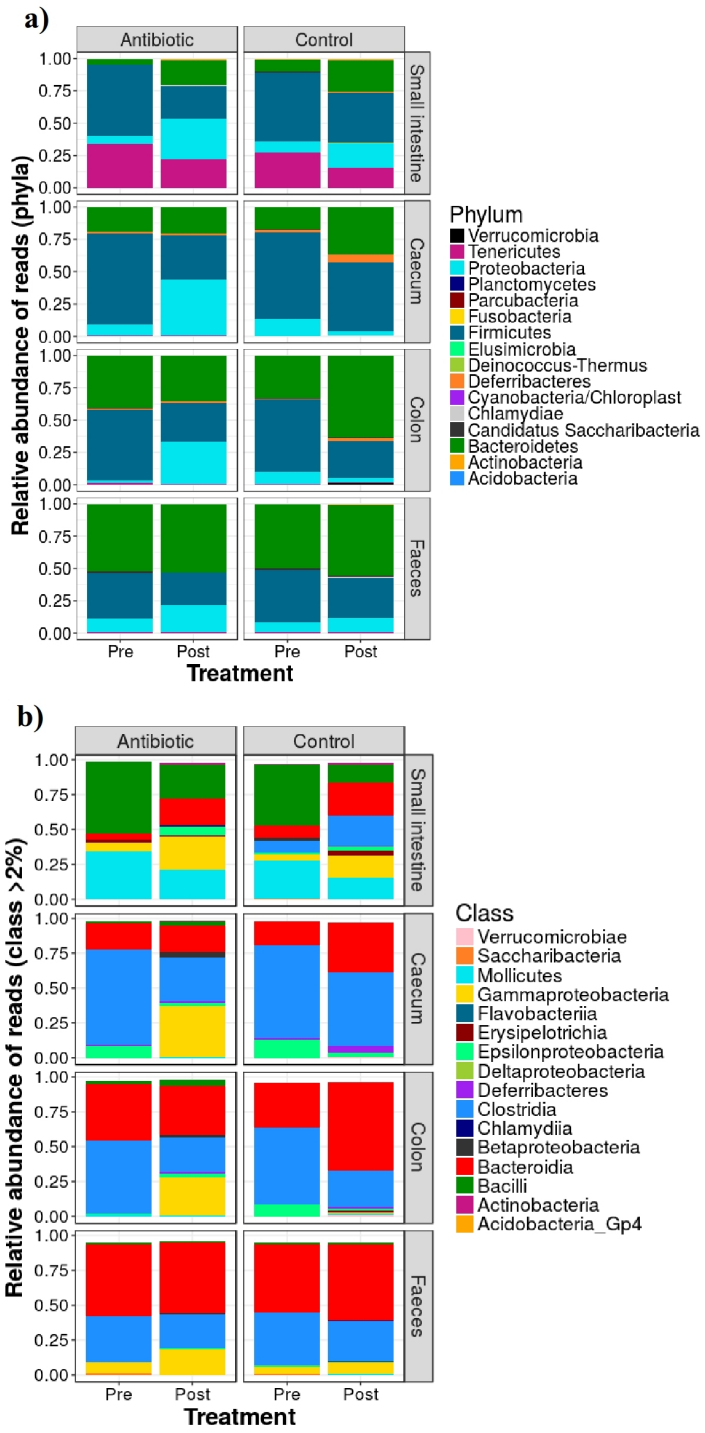

Figure 4.7: Relative abundance of reads of bacterial a) phyla and b) classes (>2%) present in different gut sections and faeces of mice pre- and post-treatment with antibiotic or a control sham gavage.

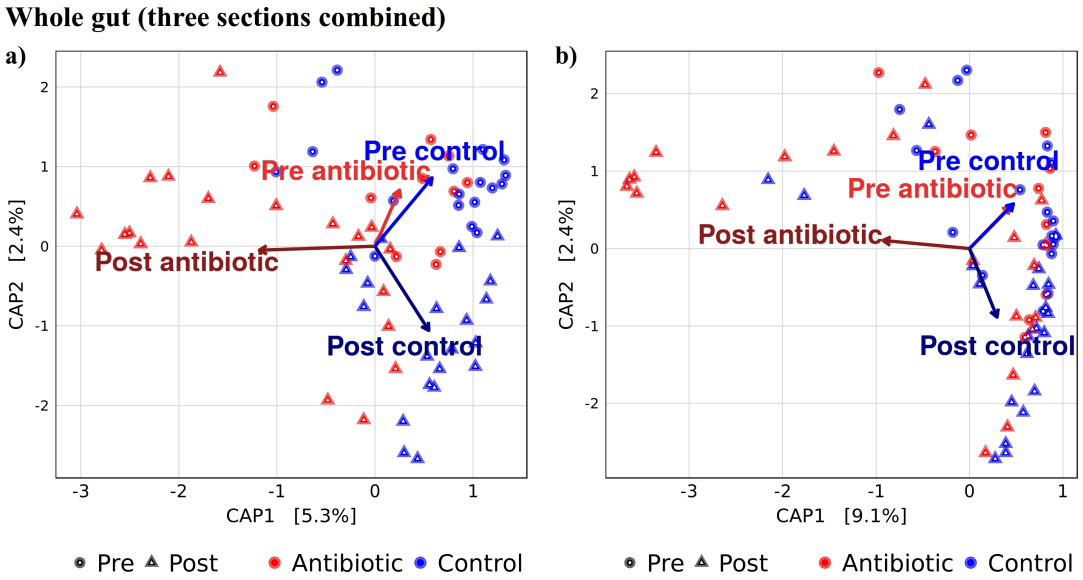

Figure 4.8: Ordination plots of divergence of microbiota taxonomic composition between samples of three gut sections (small intestine, caecum and colon) combined, associated with treatment with either antibiotic or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation are shown. The length of the arrow indicates the relative importance of each treatment.

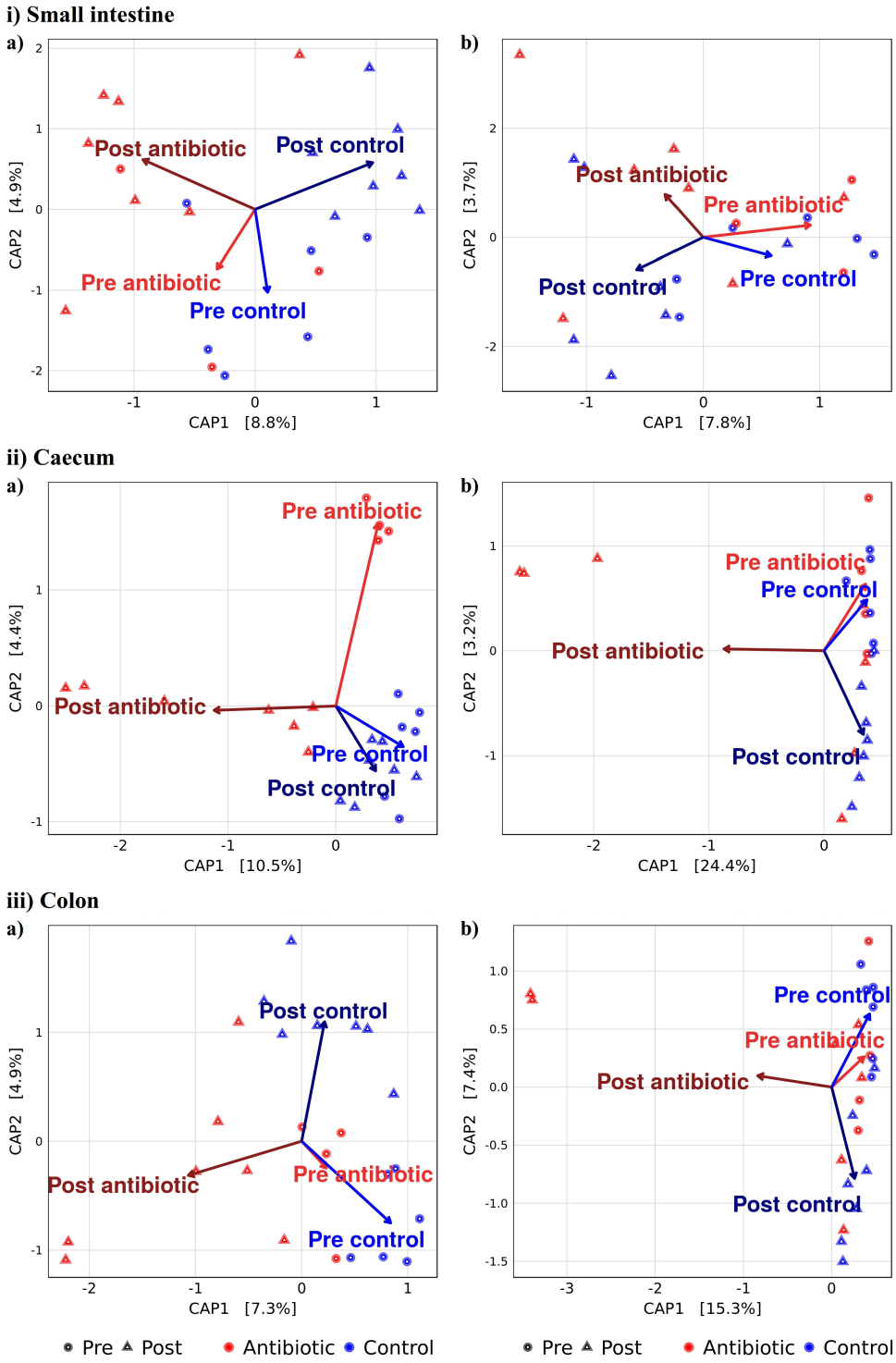

Figure 4.9: Ordination plots of divergence of microbiota taxonomic composition between i) small intestine, ii) caecum and iii) colon samples, associated with treatment with either antibiotic or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation. The length of the arrows indicate the relative importance of each treatment.

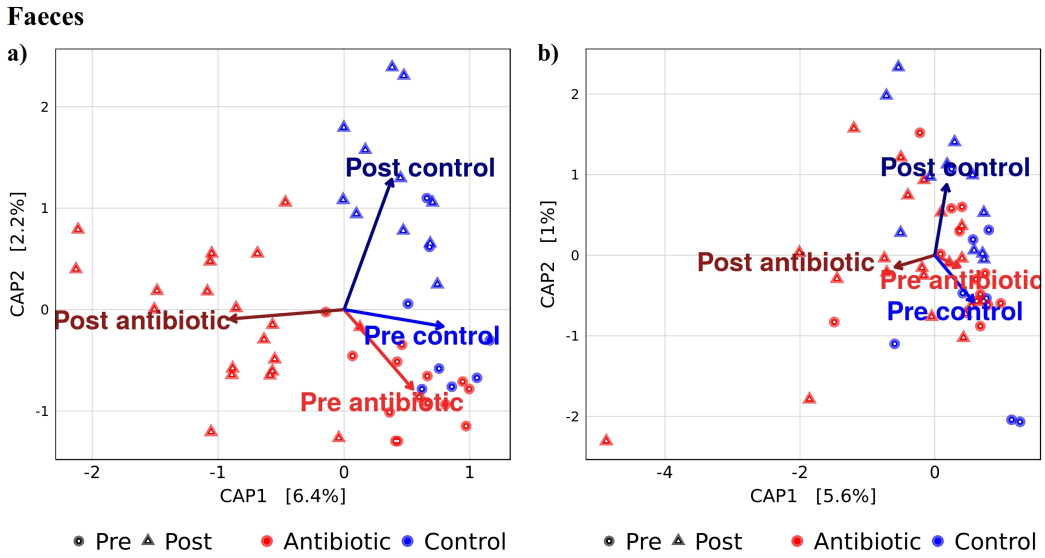

Figure 4.10: Ordination plots of divergence of microbiota taxonomic composition between faecal samples associated with treatment with either antibiotic or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation are shown. The length of the arrows indicate the relative importance of each treatment.

4.4.8 The effect of antibiotic on gut and faecal microbiota OTU abundances

Antibiotic treatment was associated with significant changes in microbial OTUs for all gut sections, and in faeces (see Appendix A.4 and tables therein for detailed statistics). *Gammaproteobacteria* and *Epsilonproteobacteria* from the *Proteobacteria* phylum consistently showed changes in abundance in post- compared to pre- antibiotic treatment individuals (Figure 4.11; Appendix A.4). OTUs from the *Firmicutes* phylum also consistently exhibited changes in abundance, generally decreasing, between pre- and post-treatment individuals, including also in the control group (Figure 4.11). Notably, in the control group *Bacteroidia* was higher in abundance in post-treatment compared to pre-treatment individuals in all gut sections, but not faeces. However, *Bacteroidia* were not significantly different in abundance between pre- and post-antibiotic treatment individuals, except in faeces, in which this bacterial class decreased in

abundance. Bacteria in four other phyla exhibited changes in abundance between pre- and post-treatment individuals in both the antibiotic and control group (Figure 4.11; Appendix A.4).

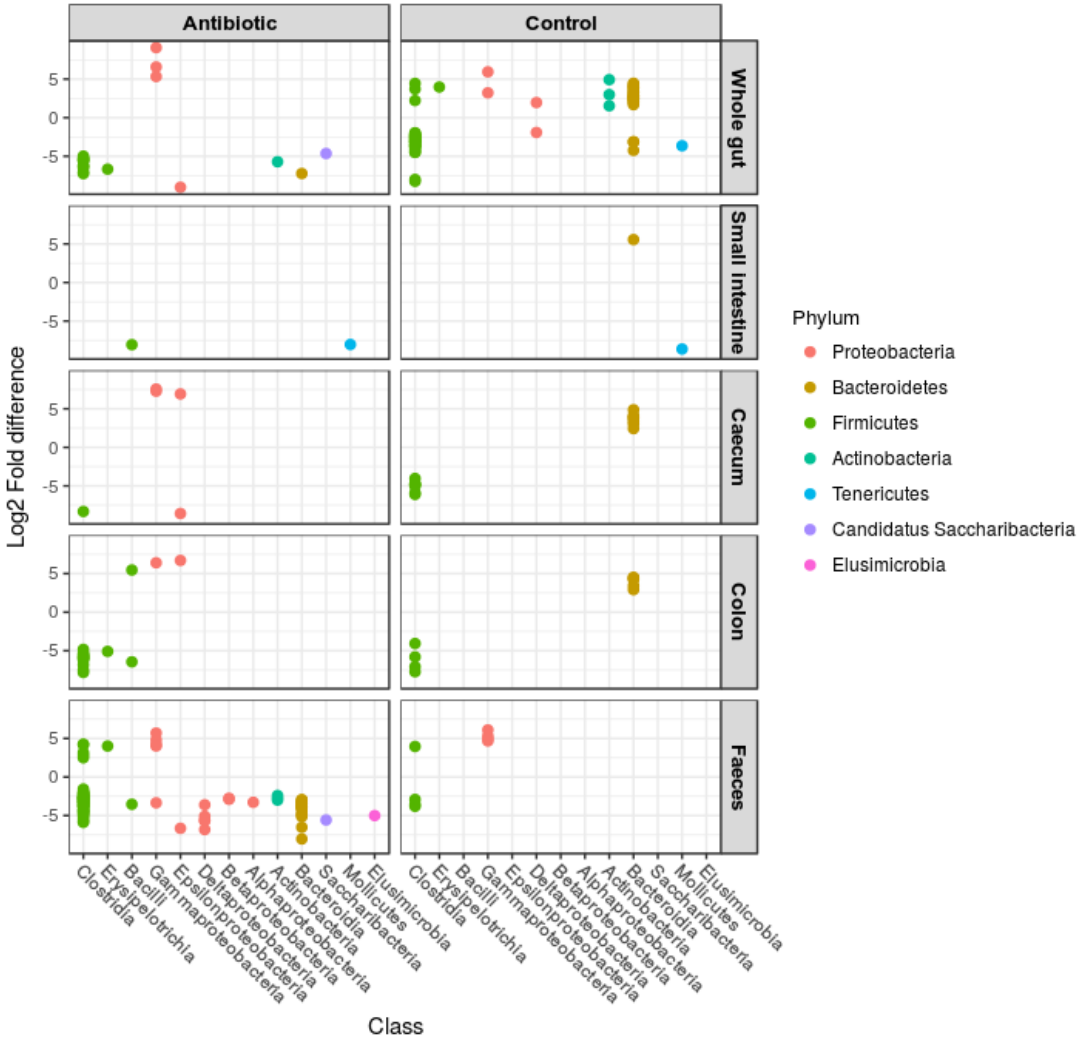

Figure 4.11: OTUs in the gut microbiota that were significantly different in abundance in post-treatment compared to pre-treatment individuals in an antibiotic treatment and control group. Microbiota of the whole gut (three gut sections combined), small intestine, caecum, colon and faeces were analysed. OTUs were grouped by microbial class. Briefly, DESeq was used to identify significantly different (p <0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing pre- and post-treatment mice.

4.5 Discussion

The present study demonstrates that antibiotic treatment does affect the helminth community, and is in general associated with significant increases in shedding of eggs in faeces, and a significant increase in the size of *Hymenolepis* spp. In addition, antibiotic treatment was associated with substantial but not significant decreases in helminth abundance, but increases in prevalence and *in utero* egg fecundity of those helminths remaining. The helminth community of the control group also exhibited changes between pre- and post-treatment individuals, none of which were significant, and were likely due to seasonal variation in helminth abundance and fecundity (linked to variables such as host breeding status and population density; Montgomery and Montgomery, 1988; Stien *et al.*, 2002; Chylinski *et al.*, 2009).

Interestingly helminth egg shedding (eggs per gram of faeces) of *H. polygyrus, Hymenolepis* and all species combined was higher in individuals treated with antibiotic, compared to pre-treatment individuals. While it has been argued that faecal egg counts are an unreliable method of establishing helminth fecundity (Michael and Bundy, 1989; Tompkins and Hudson, 1999) the differences in egg shedding observed in the current study were both substantial (362.4% for *H. polygyrus* eggs and 2,164.7% for *Hymenolepis* eggs) and significant. Although *in utero* egg counts did increase for *H. polygyrus*, they decreased *Hymenolepis*, and in both cases the differences were not significantly associated with antibiotic treatment. Thus the increase associated with antibiotic treatment observed in EPG is unlikely to have resulted from a net increase in egg production, but rather an increase in those eggs that were shed. In addition, as the percentage of female *H. polygyrus* only marginally increased following antibiotic treatment (1.7%), it is unlikely that egg shedding increased due to a higher proportion of females in the population.

Despite substantial differences in abundance and *in utero* fecundity of helminths between pre- and post-treatment individuals, antibiotic was not found to have a significant effect on any of these parameters. Previous studies from the 1950s demonstrated that antibiotic treatment of a host did have a significant negative impact on helminth prevalence and abundance (Wells, 1951, 1952a, 1952b; Chan, 1952; Salem and el-Allaf, 1969), however crude statistical analyses were used to test for these significances. In addition, these studies largely investigated the effect of antibiotic on a single helminth species in experimentally infected laboratory rodents (Wells, 1951, 1952a, 1952b; Chan, 1952; instead Salem and el-Allaf, 1969 studied human patients), thus did not take into account the effects of antibiotic on interacting coinfections of a replete helminth community (Lello *et al.*, 2004; Telfer *et al.*, 2010). For example, while antibiotics may have an effect on the abundance of a single helminth species, if this species also interacts synergistically or antagonistically with other species in the helminth community, the net effect on abundance may be reduced or exacerbated. Here, GLMMs testing the effect of antibiotic on helminth abundance included data from pre- and post-treatment individuals from the control group in an attempt to control for the seasonal variation in abundance exhibited by helminth species (Montgomery and Montgomery, 1988), however, it should be noted that the helminth community in control group stochastically differed to that of the antibiotic group. For example, control individuals harboured only three of the seven species present in the antibiotic group, which may have made statistical comparisons between these two groups erroneous. In addition, sample sizes were low, which can particularly be an issue when statistically analysing parasite data. Typically, the distribution of parasites within hosts of a population is skewed such that 20% of the host population harbour 80% of the parasites within that population (Perkins *et al.*, 2003). As such, low numbers of heavily infected individuals can have large effects on data skew and analyses.

In the antibiotic group, *H. polygyrus* isolated from the small intestine of post-treatment individuals had increased mean size and fecundity: *in utero* egg count increased by 64% and faecal egg output significantly increased by more than 360% (Figure 4.3). In addition *Bacilli* in the small intestine showed a significant decrease in abundance (Figure 4.11). Instead, in the control group *Bacilli* abundance did not significantly change, and *H. polygyrus* in post-treatment individuals had reduced prevalence, abundance, size and fecundity (although not significantly so) compared to pre-treatment mice (Figure 4.1 and 4.3). These results suggest that *H. polygyrus* has improved fitness when *Bacilli* abundances are lower. Indeed *Bacilli* bacteria have been touted as potential anthelmintics as they prevent egg production and larval development of nematodes, often leading to death (Charles *et al.*, 2005; Kotze *et al.*, 2005). Removing *Bacilli* from the host gut using antibiotic appears to release the helminth from the fitness constraints imposed by this bacteria, allowing helminth fitness to increase.

Interestingly, helminths can interact with bacteria up or downstream from the gut niche that they inhabit (Rausch *et al.*, 2013; Kreisinger *et al.*, 2015; McKenney *et al.*, 2015). For example, *Hymenolepis* spp*.,* which generally infect the small intestine, have been associated with an increase in *Clostridia* bacteria in the caecum (McKenney *et al.*, 2015). In the present study, *Hymenolepis* spp. abundance decreased, by nearly 87% (although this change was not significant) following antibiotic treatment (Figure 4.1), while *Clostridia* in the caecum also decreased (Figure 4.11). The directionality of the observed relationship is not clear, however, *Clostridia* have been associated with dysbiosis (Winter and Bäumler, 2014), a bacterial imbalance in the gut which can lead to disease, and could potentially make individuals more susceptible to other infections (e.g., helminth infection). Likewise, high abundances of tapeworm species can predispose individuals to pathogenic *Clostridia* infection, perhaps due to the fact that the immune system cannot effectively

respond to both a macro- and microparasite infection simultaneously (Elliott, 1986; Uzal, 2004). It should also be noted that the decrease in *Hymenolepis* spp. abundance may have been responsible for the increase in fecundity of this species; due to competitive release for resources, those remaining *Hymenolepis* may have higher fitness and be able to produce more eggs (Dezfuli *et al.*, 2002; Lagrue and Poulin, 2008).

The specific changes in microbiota following antibiotic or control treatment may not account for all observed changes in the helminth community resulting from treatment. Antibiotic treatment can reduce bacterial loads by 10^6 –10^7 for anaerobic and 10^5 –10^6 for aerobic bacteria, opening up attachment sites and nutrient availability within the gut for helminths to acquire (Zaiss *et al.*, 2015). As such, reduction in microbiota following antibiotic treatment may leave the host more susceptible to parasite infection. Indeed, in the current study prevalence of both *H. polygyrus* and *Hymenolepis* increased post-antibiotic treatment. Antibiotic may also have effected helminths by indirectly affecting crucial bacterial symbionts within the host gut. For example, some helminth species may rely on bacteria to digest nutritional substrates (Biswal *et al.*, 2016), or to complete their life-cycle (Hayes *et al.*, 2010), and these bacteria may be affected by antibiotic treatment, with a knock-on effect on helminths. In addition, the current study did not account for changes in the microbiota of the helminths themselves. Evidence has shown that both free-living and parasitic nematodes can harbour a microbiota (Tan and Grewal, 2001; Lacharme-Lora *et al.*, 2009; Diaz and Restif, 2014; see also Perkins and Fenton, 2006 and Chapter 5), and some nematodes even rely on symbiotic bacteria such as *Wolbachia* to survive, and die when the bacteria is removed by antibiotic (Saint André *et al.*, 2002; Taylor *et al.*, 2005). Consequently, antibiotic treatment may have influenced helminth fitness by effecting symbiotic bacteria in the helminth microbiota.

Interestingly, the antibiotic combination that was administered here to wild mice has been associated with altered expression of 517 different genes in the epithelium of the colon (Reikvam *et al.*, 2011). Of note, the genes *Ang4, Retnlb, Reg3g, Reg3b, Pla2g2a* and *Pla2g4c* have all previously shown a substantial decrease in expression following antibiotic treatment (Reikvam *et al.*, 2011). These genes normally show an increase in expression following helminth infection, with some also demonstrating anthelmintic properties (Artis *et al.*, 2004; Nair *et al.*, 2008; D'Elia *et al.*, 2009; Forman *et al.*, 2012; Hurst and Else, 2013; Weinstock and Elliott, 2014; Fricke *et al.*, 2015). For example, *Retnlb* may impair chemosensory activity of the nematode *Strongyloides stercoralis* (Artis *et al.*, 2004). As expression of these genes which are linked to anthelmintic activities decrease after antibiotic treatment, individuals may be subsequently more susceptible to helminth infection, and could explain why an increase in helminth prevalence and fecundity were observed here.

In summary, antibiotic treatment does affect the helminth community, and is most notably associated with a significant increase in helminth egg shedding, and size of *Hymenolepis* spp. In addition antibioitc treatment is associated with non-significant decreases in helminth abundance, but increases in helminth prevalence and fecundity. Increased prevalence of helminths following antibiotic treatment may be a knock-on effect associated with an increase in helminth fecundity following antibiotic administration. Release from resource competition and/or immune-mediated interactions, removal of bacteria which interact with helminth fitness, and changes in gene expression associated with antibiotic treatment in host genes which are involved in protecting against helminth infection may have incited changes in the helminth community (e.g., Hayes *et al.*, 2010; Reikvam *et al.*, 2011; Biswal *et al.*, 2016). The work presented here suggests that while antibiotic may at first appear an effective method of treating helminth infection, as in the short-

term helminth abundance may be reduced, long-term implications could include more fecund helminths leading to higher rates of transmission in the population.

4.6 Author Acknowledgements

The manuscript resulting from this chapter is authored by:

Emily L. Pascoe, Jakub Kreisinger (Charles University, Prague), Margherita Collini (Fondazione Edmund Mach), Heidi C. Hauffe (Fondazione Edmund Mach), Julian R. Marchesi (Cardiff University and Imperial College) and Sarah E. Perkins (Cardiff University and Fondazione Edmund Mach).

Chapter 5

Composition and diversity of the microbiota of parasitic helminths

"An understanding of the natural world and what's in it is a source of not only a great curiosity but great fulfilment."

David F. Attenborough

5.1 Abstract

Microbiota and parasitic helminths have co-evolved within the gut for millennia. A burgeoning research area currently focuses on the two-way interactions between helminths and host gut microbiota. The microbiota of the helminth itself, however, warrants investigation, as it may play a role in microbiota-macrobiota interactions. Here, the diversity and taxonomic composition of microbiota associated with six parasitic helminth species from wild, naturally infected rodents, and the gut niche in which they were co-located, are quantified. Helminth microbiota differed in terms of alpha diversity, taxonomical composition and OTU abundances between, and within species. *Heligmosomoides polygyrus* were associated with the most taxonomically rich microbiota: 257 different genera were identified across this species, of which one sample was associated with 133 genera. However, mean alpha diversity was instead highest in *T. muris* (33.0 ± 4.3 standard error). For all but one species (*Mastophorus muris*), alpha diversity of the helminth microbiota exceeded that of the gut section in which the helminth was found, on at least one occasion. Samples from three helminth species were associated with a monoculture microbiota, 50.5% of sequenced samples of *Hymenolepis diminuta,* 12.5% *Aonchotheca murissylvatici* and a single *M. muris* were associated with a single OTU constituting ≥99% microbiota, including genera encompassing putative pathogenic bacteria. Intraspecific variation in composition of helminth microbiota was significantly driven by the inhabitation of different gut sections, for all species except *H. diminuta*. Taxonomic composition of helminth microbiota significantly differed to that of the gut microbiota for any given gut location, suggesting that bacterial colonisation of helminths is non-random, and bacteria may be acquired from other sources, e.g., an intermediate host or the environment. These data provide the first steps in identifying microbes associated with helminths that are potentially crucial for helminth survival. In the future, symbiotic bacteria important for helminth survival could be targeted for removal (e.g., by antibiotic) as a novel

method by which to control helminth infection.

5.2 Introduction

The number of studies on microbiota have rapidly increased in the last decade (Marchesi and Ravel, 2015), and although research on this topic initially focussed on microbial communities of the human gut, the microbiota of non-human animals is now also a burgeoning area of research (see Chapter 2). The gut microbiota in particular has revealed itself to have so many important functions within the host that it has earned the accolade 'the undiscovered organ' (Bäckhed *et al.*, 2005). Ubiquitous across species, and numerous in the gut are parasitic helminths, causing considerable morbidity in both humans and animals (Huffman and Seifu, 1989; Chan, 1997; Hotez *et al.*, 2008; Shetty, 2010; Sutherland and Scott, 2010; Morgan *et al.*, 2012. Previous work has shown that helminths can be associated with bacteria (Taylor *et al.*, 2005; Lacharme-Lora *et al.*, 2009b; Walk *et al.*, 2010), and in some cases vector pathogenic bacteria to the host (Perkins and Fenton, 2006). Given that helminths can be parasitic, the composition and diversity of microbiota that they are associated with could have implications for host health. Studies on parasite microbiota have thus far mostly focussed on the biting ectoparasitic arthropods, such as fleas (Jones *et al.*, 2013), ticks (Carpi *et al.*, 2011), tsetse flies (Weiss *et al.*, 2013) and mosquitoes (e.g., Dong *et al.*, 2009; Chandel *et al.*, 2013), no doubt due to their vectorial capacities. Insight into the bacterial composition of parasites may lead to an avenue for their control; for example, symbiotic bacteria crucial for pathogen or parasite survival could be targeted for removal by targeted antibiotics, to indirectly prevent pathogen transmission or kill the parasite vector (Taylor *et al.*, 2005; Ramirez *et al.*, 2012; Minard *et al.*, 2013).

Tantalising evidence that helminths harbour a microbiota, and that this microbial community plays a function in helminth health does exist. Previous work has shown that administration of antibiotics to humans and mice causes a decrease in helminth burden and prevalence, and helminths that remain after treatment are reduced in size, fecundity and virulence (Wells, 1951, 1952a, 1952b; Brown, 1952; Chan, 1952; Salem and el-Allaf, 1969; Hoerauf *et al.*, 1999; Saint André *et al.*, 2002; But see Chapter 4 in which fecundity of helminths was increased after antibiotic treatment). However, these early studies were unsure why antibiotic treatment had this effect on helminths, nor if any bacteria were associated with the helminths themselves, which could have also been effected by the antibiotic leading to decreases in helminth abundance. Although these studies do not represent evidence of a helminth microbiota, they do imply that antibiotics may kill or reduce symbiotic bacteria crucial for helminth growth and survival. One known case of a helminth-bacteria symbiosis is with *Wolbachia* spp., which within helminths appears to be found strictly within filarial nematodes (Taylor *et al.*, 2005; Duron and Gavotte, 2007; Foster *et al.*, 2014). Antibiotics that target *Wolbachia* spp. can reduce or eliminate certain filarial infections (Bandi *et al.*, 2001; Taylor *et al.*, 2005. Microscopy has identified vertically transmitted micro-organisms within helminth tissue, e.g., the hypodermis, which may affect helminth development (Mclaren *et al.*, 1975; Kozek and Marroquin, 1977; Franz and Büttner, 1983). However, bacterial infections have largely been observed in free-living nematodes, and only rarely in parasitic species (Mclaren *et al.*, 1975; Kozek and Marroquin, 1977; Franz and Büttner, 1983), but this lack of evidence may well be due to a lack of observations.

One of the few studies on helminth microbiota demonstrated that bacteria are present within infective (but free-living) L3 larvae of the common livestock parasitic helminths *Ostertagia ostertagi*, *Cooperia onchophora* and *Haemonchus contortus*, which are most likely acquired from

the host faeces in which they develop (Lacharme-Lora *et al.*, 2009b). Notably, this study utilised culture-dependent techniques, which are unlikely to have identified the full consortia of bacteria associated with the parasites, since not all bacteria in the microbiota can be cultured (Suau *et al.*, 1999). More recently, culture-independent techniques have shown that the non-parasitic nematode *Caenorhabditis elegans* harbour a core microbiota, regardless of its external microbial environment (Berg *et al.*, 2016), although environment, as well as the developmental stage and genetics of the helminth do have a role in shaping *C. elegans* microbiota composition (Berg *et al.*, 2016; Dirksen *et al.*, 2016). Microbiota associated with parasitic nematodes has been described using culture-independent techniques, but in just one study; laboratory strains of infective L3 *H. polygyrus* are associated with a unique, but depauperate, microbial community, while adult *H. polygyrus* recovered from experimentally infected laboratory mice had a microbial composition similar to that of the mouse ileum that they infected (Walk *et al.*, 2010). However, few bacterial sequences were recovered from *H. polygyrus* in this study (Walk *et al.*, 2010), and as observed in other taxa it is possible that the microbiota of laboratory-derived helminths is reduced in diversity in comparison with wild-type individuals (Amato, 2013; Wang *et al.*, 2014). The microbiota of enteric helminths has yet to be investigated in a wild, naturally infected system.

Understanding the composition of microbiota associated with parasitic helminths has multiple implications. As demonstrated by studies on *C. elegans*, characterising the microbiota of parasitic helminths is the first step to understanding helminth-microbe interactions, which in the future could lead to identification of bacteria crucial to helminth survival or fitness, that could be targeted in parasite control strategies (e.g., by using antibiotic). Previous studies have already demonstrated that helminth infections can be controlled by treating the host with antibiotic (Wells, 1951, 1952a, 1952a; Brown, 1952; Chan, 1952; Salem and el-Allaf, 1969; Hoerauf *et al.*, 1999;

Taylor *et al.*, 2005; Hayes *et al.*, 2010; Biswal *et al.*, 2016), but a more targeted and informed approach, e.g., using narrow-spectrum antibiotics which affect just those bacteria associated with the helminth and not the host, could reduce negative repercussions (such as a dysbiotic microbiota) for the host. Given that studies using microscopy, and both culture-dependent and -independent methods have found initial evidence of a helminth microbiota, it is timely that it should be investigated and quantified more comprehensively in a wild, replete system. Here, the diversity and composition of microbiota associated with helminths isolated from naturally infected wild mice (*Apodemus flavicollis*), and the gut location from which they were found is quantified, in order to ask the questions 'what is the microbiota composition and diversity of a helminth community?' and 'is the helminth microbiota unique to that of the host?'.

5.3 Materials and Method

5.3.1 Sample collection

Thirty-two adult *A. flavicollis* (14 females and 18 males) were live-trapped from April to July 2015 in deciduous woodland habitat at San Michele all'Adige (46°11'24.8"N, 11°08'27.6"E) and at Lagolo, Monte Bondone (46°03'28.6"N, 11°00'47.9"E), in the Province of Trento, Italy. Animals were euthanised by an overdose of isoflurane, followed by cervical dislocation. The following steps were performed under sterile conditions. The entire digestive tract was dissected from the animal and submerged in Tris-buffered saline (TBS; 50 mM Tris, 200 mM NaCl, pH8). Following external washing with TBS, the digestive tract of each mouse was divided into five sections: stomach, small intestine, caecum, proximal colon and distal colon. The membrane and luminal contents of each gut location were diluted with TBS and scanned for parasitic helminths under a Leica MS5 stereomicroscope (Leica Microsystems, Wetzlar, Germany), at 10×

magnification. Helminths from a single individual were collected in TBS according to species and the gut location from which they were isolated (herein referred to as a 'sample' of helminths, see Appendix A.5, Table A.5.1, A.5.2 and A.5.3 for details) and quantified. Additionally, at this stage any host gut membrane or luminal content attached to any helminth was manually removed using sterile tweezers. External debris was removed from each sample of helminths by transferring to a 20 μm pore cell strainer and washing with 50 ml of TBS four times. Helminth samples were then stored at -80°C for future DNA extraction (see '*5.3.2 16S rRNA gene sequencing*' below). After thoroughly scraping the gut membrane with tweezers under TBS to dislodge bacteria, the membrane and the TBS containing bacteria were collected with the rest of the luminal contents in a centrifugation tube. A bacterial pellet was obtained by centrifugation (950 G for 10 minutes at 4°C, resulting supernatant 9,000 G for 15 minutes at 4°C. The membrane did not form part of the pellet during the second centrifugation and was discarded). The bacterial pellet was immediately stored at -80°C for future bacterial DNA analysis (see '*5.3.2 16S rRNA gene sequencing*' below).

5.3.2 16S rRNA gene sequencing

Preliminary analyses showed that low quantities of DNA were recovered from single helminths of some species (data not shown). Thus, DNA extraction was performed on helminth samples that had previously been pooled according to species, gut location, and individual mouse from which they had been isolated (See Figure 5.1 and Appendix A.5, Table A.5.1, A.5.2 and A.5.3 for details). Consequently, due to natural variation in helminth prevalence and abundance between hosts, the number of individual helminths varied per samples (see Figure 5.1 and Appendix A.5, Table A.5.3 for helminth sampling details). Total genomic DNA was extracted from 273 samples (115 gut sections and 158 helminth samples; composed of 2,091 individual helminths) using the QIAamp DNA Micro kit (Qiagen, Valencia, CA, USA), following manufacturer methods for the

isolation of genomic DNA from tissue, with the addition of carrier RNA. Purity and quality of the recovered DNA were determined using a QIAxcel capillary electrophoresis system (Qiagen, Valencia, CA, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified using the primers 341F and 805R, with Illumina Adapters included (see Appendix A.2, Figure A.2.1 for details on primer sequences, including degenerate nucleotides), and sequenced using a 2×300 bp kit on the Illumina MiSeq system (Illumina, San Diego, CA, USA). Polymerase chain reactions (PCRs) were carried out in a total volume of 25 µl with 0.2 µM of each primer, 1.5 µl of 2× KAPA HiFi HotStart ReadyMix and 1.5 ng (gut sections) or 25 ng (helminths) of template DNA. Thermal cycling was performed on a GeneAmp™ PCR System 9700 instrument (Thermo Fisher Scientific, Waltham, MA, USA) as follows: initial denaturation at 95°C for 5 minutes, followed by 28 (gut sections) or 35 (helminth samples) cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. Negative controls were included, and genomic DNA from a Microbial Mock Community B (Staggered, Low Concentration), v5.2L (BEI Resources, Manassas, VA, USA) was included to assess the effect of data processing on observed community content. PCR amplification results were checked by agarose gel electrophoresis and purified from free primers and primer dimer species using AMPure XP beads (Beckman Coulter, USA). Dual indices and Illumina sequencing adapters were attached using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). The final library was cleaned, quantified, normalised and pooled in an equimolar way before sequencing at the Illumina platform at the University of Trento, Trento, Italy. Sequencing was carried out following the manufacturer's recommendations.

5.3.3 Bioinformatic processing of 16S data

Sequences were merged, trimmed and filtered using MICCA software (version 1.5.0, Albanese *et*

al., 2015). Overlapping regions of the forward and reverse read sequences that differed by more than eight nucleotides, or did not contain both the forward and reverse PCR primer sequences were discarded. The resulting merged 16S rRNA fragments were discarded if they had an average expected error (AvgEE) greater than 0.23. OTUs were assigned using a *de novo*, greedy strategy with a cut-off of 97% similarity based on the VSEARCH clustering algorithm (Rognes *et al.*, 2016). Chimeric samples were discarded. Resulting representatives of each OTU were classified using the Ribosomal Database Project classifier (RDP classifier, version 2.12; Michigan State University [http://rdp.cme.msu.edu/]). Samples that had final read counts of less than 2,000 merged and quality-filtered reads were discarded. The resulting OTUs were analysed using Phyloseq version 1.16.2 (McMurdie and Holmes, 2013).

5.3.4 Statistical analyses of microbiota - composition

For helminth species isolated from multiple gut locations, distance-based redundancy analyses (db-RDA; capscale function in R package Phyloseq) were used to test for taxonomical differences in microbiota composition within a helminth species associated with inhabitation of different gut locations. In addition Db-RDA analyses were used to test for taxonomical differences in microbiota composition between a given gut section (stomach, small intestine, caecum, proximal colon or distal colon) and the helminth species therein. Ecological distances between microbiota taxonomy were assessed using Bray–Curtis dissimilarities (i.e., compositional dissimilarity index that accounts for proportional differences of OTUs among samples), and weighted UniFrac distances (which account for both proportional differences of OTUs and their phylogenetic relatedness; Lozupone and Knight, 2005). OTU tables were scaled before calculation of dissimilarity matrices, to achieve an even sequencing depth corresponding to a minimal number of reads per sample in gut sections and helminths included in any given analysis. Significance was

assessed using permutation-based marginal tests.

5.3.5 Statistical analyses of microbiota – OTU abundances

To determine how OTUs varied for a given helminth species isolated from different gut sections, differentially abundant OTUs (i.e., number of reads corrected for sequencing depth) were identified using an approach based on generalised linear models with negative binomial errors (Anders and Huber, 2010). These analyses were conducted using the default pipeline set-up in DESeq2, and significance values (p >0.05) were derived using likelihood-ratio tests. Analyses were performed using the DESeq 2 package, version 1.14.1 (Love *et al.*, 2014).

5.4 Results

5.4.1 What is the diversity and composition of the helminth microbiota?

From 32 mice, a total of six helminth species were identified, five species of nematode: *Aonchotheca murissylvatici, Heligmosomoides polygyrus, Mastophorus muris, Syphacia frederici* and *Trichuris muris,* and one cestode species: *Hymenolepis diminuta* (Figure 5.1). With the exception of *H. polygyrus,* which were found only in the small intestine, and *T. muris,* which were only found within caeca samples, each helminth species infected multiple locations in the gut (Figure 5.1; Appendix A.5, Table A.5.3). Prevalence and abundance (total number of helminths, including zero values of uninfected hosts, as defined by Bush *et al.*, 1997) of each helminth species varied and not every species infected every individual (Table 5.1; Appendix A.5, Table A.5.2). The filtered dataset consisted of 5,956,246 high-quality reads from 115 gut samples (mean ± standard error = 20,221 ± 724, range = 3,966-39,769). Sequences from one distal colon sample did not meet the quality filtering criteria and were excluded from analyses. In addition, 158

helminth samples, equating to 2,091 individual helminths were sequenced (see Appendix A.5, Table A.5.3 for details of helminth sampling, see Table 5.1 for mean number of reads obtained from each species, range = 2,228 - 42,980). Note, sequences from two samples of *S. frederici,* composed of one helminth each, did not meet the quality filtering criteria and were excluded from analyses. The mean number of reads per sample of helminth species varied between 16,949 – 22,711, with *H. polygyrus* having fewest mean reads per sample and *M. muris* the highest (Table 5.1). The number of reads yielded from *A. murissylvatici* varied the most (19,258 ± 13,692), but the number of reads from *T. muris* remained most consistent (18,022 ± 6,764; Table 5.1).

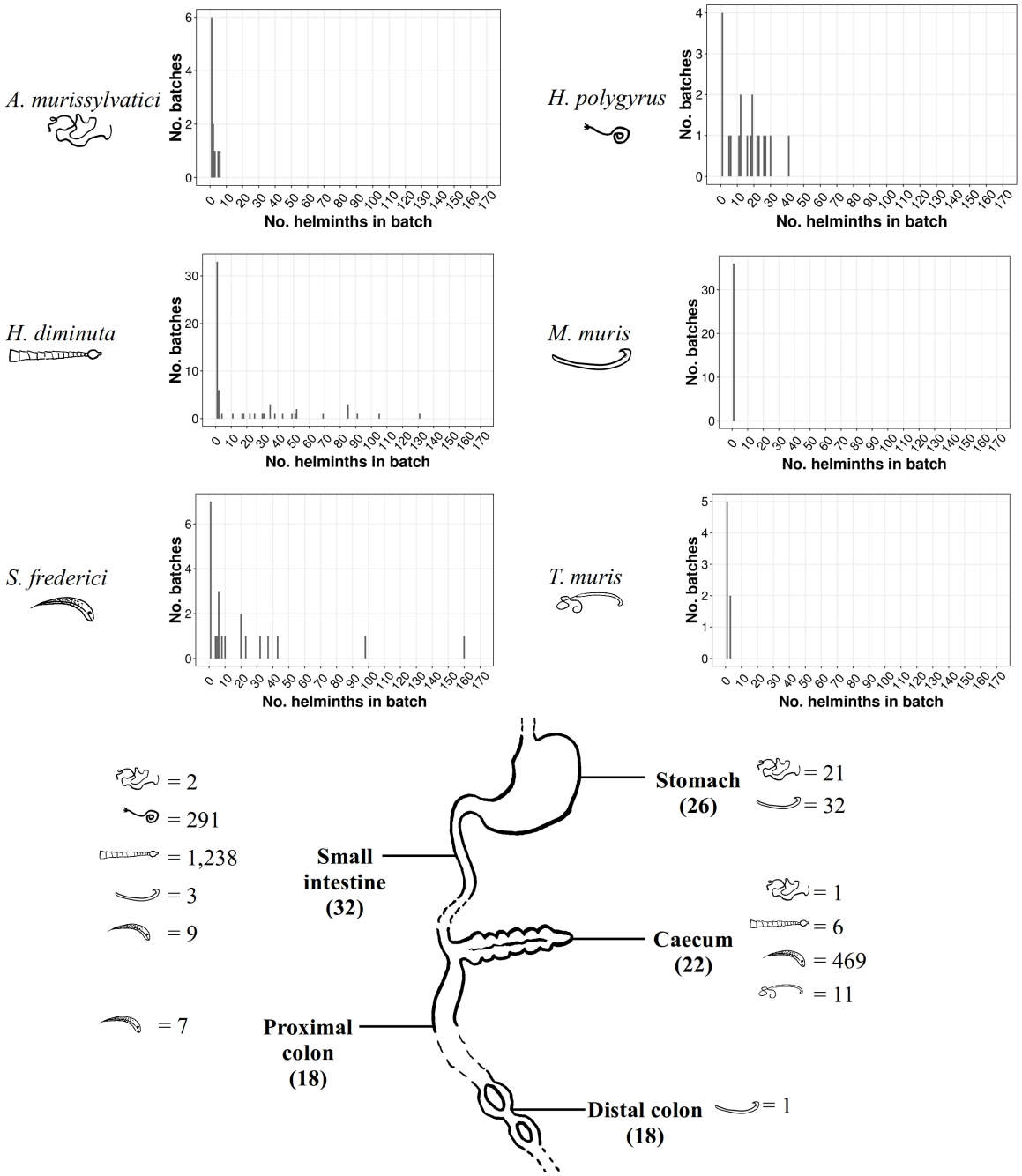

Figure 5.1: Helminth and gut samples that were sequenced from 32 *Apodemus flavicollis* individuals for analysis of microbiota diversity and composition. Microbiota were analysed from six helminth species; *Aonchotheca murissylvatici, Heligmosomoides polygyrus, Hymenolepis diminuta, Mastophorus muris, Syphacia frederici* and *Trichuris muris*. Bar charts illustrate the number of individual helminths per sequenced sample. The number of helminth individuals is indicated for each helminth species in each gut section. In addition, the microbiota of five gut locations were sequenced; stomach, small intestine, caecum, proximal colon and distal colon. Numbers in brackets below each gut section indicate how many samples of that gut section were sequenced.

Table 5.1: The prevalence and abundance of six helminth species isolated from 32 *Apodemus flavicollis,* which were sequenced for bacterial analyses. Total number of helminth individuals sequenced and the mean number of 16S rRNA reads yielded from samples of each species (± standard error of mean) are presented.

Species	Prevalence	Mean abundance	Total number of individuals analysed	Mean number of reads/sample ± SEM
A. murissylvatici	53.1%	2.2	24	19,258 ± 13,692
H. polygyrus	87.5%	11.4	291	16,949 ± 8,659
H. diminuta	96.9%	41.0	1,244	18,739 ± 7,037
M. muris	15.6%	1.1	36	22,711 ± 9,224
S. frederici	53.8%	15.8	485	17,831 ± 8,070
T. muris	21.9%	0.3	11	18,022 ± 6,764

Across all helminth species the dominant phyla (>10% reads) were *Tenericutes, Firmicutes* and/or *Proteobacteria,* but each were found in varying percentages between different helminth species (Table 5.2; Figure 5.2 and 5.3). The exception to this pattern was *S. frederici,* for which 38.0% of sample reads belonged to the phyla *Deferribacteres* and 13.9% to *Bacteroidetes* (Table 5.2; Figure 5.2 and 5.3). Intraspecific variation was observed in alpha diversity; microbiota associated with *H. polygyrus* showed the most intraspecific variation in terms of genera richness; between 15 – 133 genera were identified in this species, compared to *T. muris,* in which 31 – 71 genera were identified across samples (Table 5.2). With the exception of *T. muris* (d.f. = 5, $S = 12$, $p = 0.03$), the number of helminths within a sequenced sample did not affect alpha diversity (see Appendix Table A.5.3). Multiple samples from two helminth species were associated with a monoculture microbiota (here defined as one OTU composing ≥99% of reads); 50.5% of *H. diminuta* samples were a monoculture; 32.7% were dominated by *Tenericutes: Bacilli,* (of which 73.3% which were from the genus *Mycoplasma*), 17.4% by *Proteobacteria: Gammaproteobacteria* (87.5% genus *Escherichia/Shigella*) and 0.3% by *Firmicutes: Lactobacillus*. In addition, 12.5% of *A. murissylvatici* samples hosted a monoculture of either *Tenericutes:* genus *Mycoplasma* (8.3%) or

Proteobacteria: Gammaproteobacteria (4.2%). A single *M. muris* (1/36 sequenced) isolated from the distal colon was a monoculture of *Escherichia/Shigella.*

Table 5.2: The dominant bacterial phyla (>10% reads) and diversity of microbiota associated with six helminth species that were isolated from the guts of 32 *Apodemus flavicollis*. Dominant phyla that constituted >10% of total mean reads are presented, as are the number of bacterial classes and genera associated with each helminth species, and range of genera present across samples of a species. Mean inverse Simpson index ± standard error of mean are provided.

Species	**Dominant phyla (% reads)**	**Class diversity across samples (genera)**	**Range of genera/sample**	**Mean inverse Simpson index ± SEM**
A. murissylvatici	*Firmicutes* (50.4%), *Proteobacteria* (37.4%)	28 (137)	11 - 54	4.9 ± 1.8
H. polygyrus	*Tenericutes* (44.2%), *Proteobacteria* (22.2%), *Firmicutes* (21.5%)	38 (257)	15 - 133	5.6 ± 2.1
H. diminuta	*Tenericutes* (50.7%), *Proteobacteria* (31.9%), *Firmicutes* (12.1%)	28 (180)	4 - 50	1.9 ± 0.3
M. muris	*Proteobacteria* (55.2%), *Firmicutes* (34.3%)	26 (164)	10 - 56	2.3 ± 0.3
S. frederici	*Deferribacteres* (38.0%), *Firmicutes* (31.9%), *Proteobacteria* (14.8%), *Bacteroidetes* (13.9%)	29 (188)	19 - 96	10.5 ± 2.9
T. muris	*Firmicutes* (80.4%)	22 (113)	31 - 71	33.0 ± 4.3

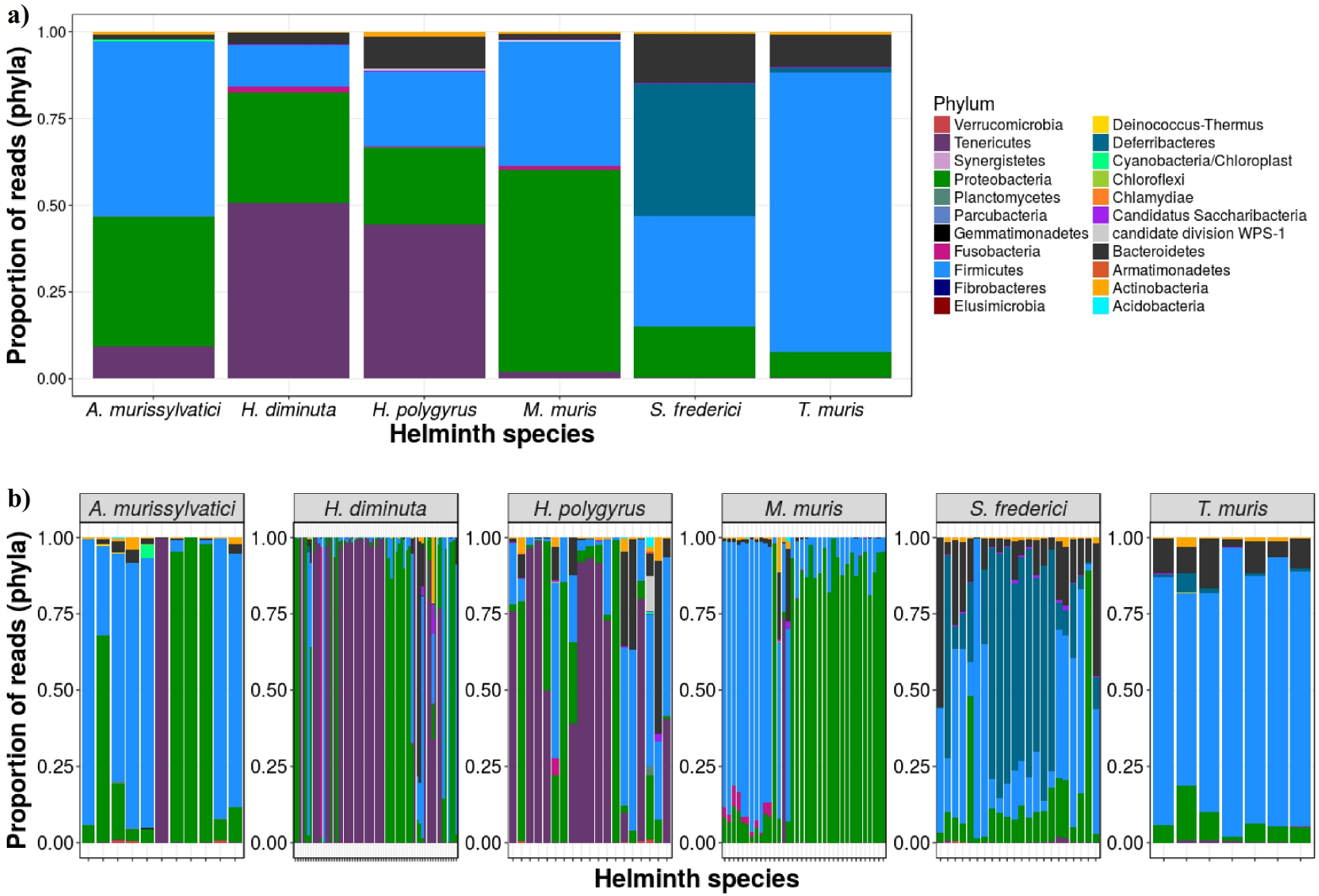

Figure 5.2: Proportion of reads composed of different bacterial phyla in microbiota associated with a) six helminth species and b) individual samples composed of either a single helminth or pooled individuals (according to gut location and individual from which the helminths were isolated) for each of the six helminth species. Helminths were isolated from 32 *Apodemus flavicollis*.

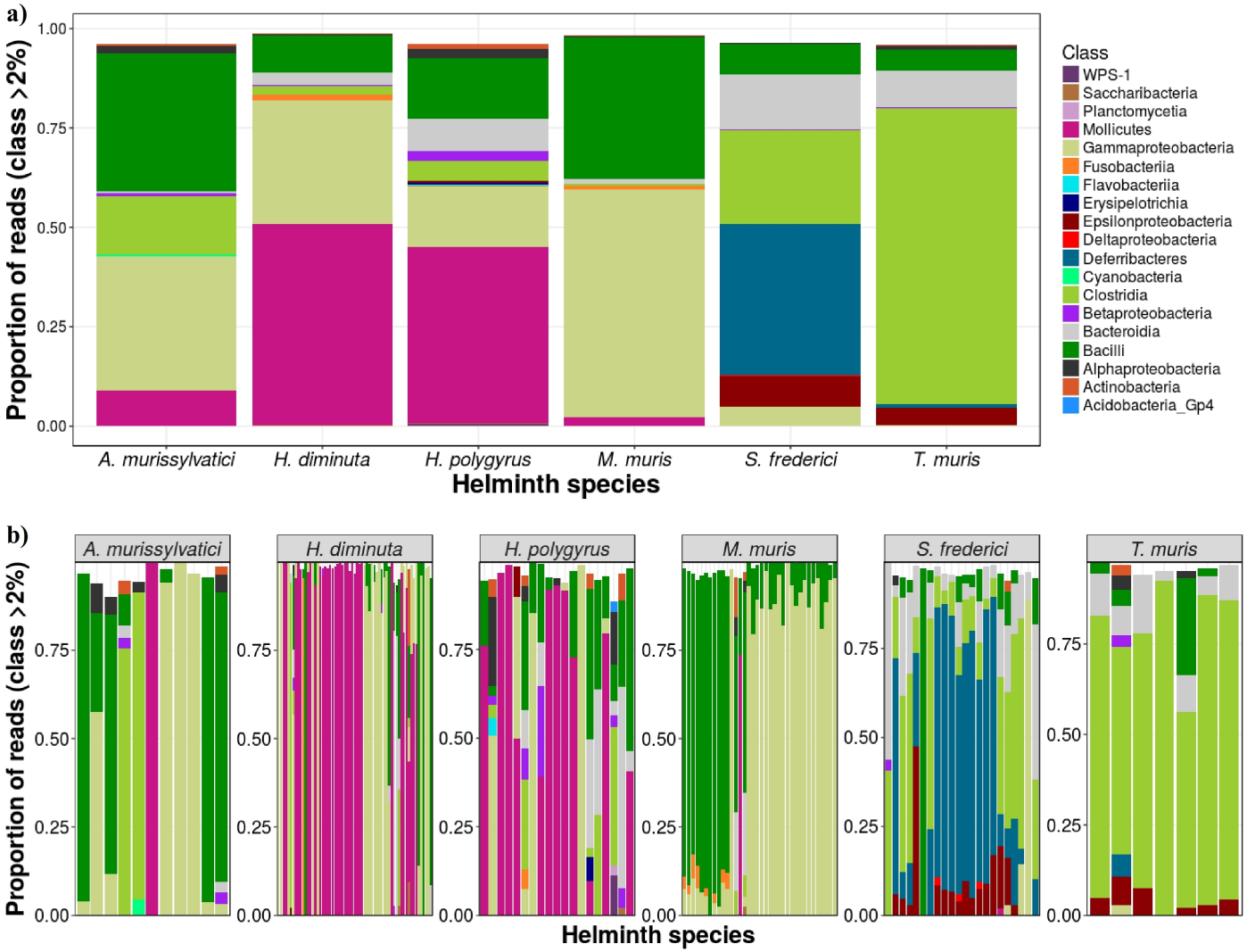

Figure 5.3: Proportion of reads (composing >2% of the total) of different bacterial phyla in microbiota associated with a) six helminth species and b) individual samples composed of either a single helminth or pooled individuals (according to gut location and individual from which the helminths were isolated) for each of the six helminth species. Helminths were isolated from 32 *Apodemus flavicollis*.

Microbiota species richness was highest in *H. polygyrus*; across all 19 samples of this species 257 genera from 38 classes were identified (Table 5.2). Microbiota of *T. muris* was the least rich, and was composed of 113 genera from 22 bacterial classes across samples, but alpha diversity was significantly higher than for any other species (33.0 ± 4.3; d.f. = 273, $Z = 2.18$, $p = 0.03$; Figure 5.4, Table 5.2). Despite being the smallest in size of all helminths identified, the highest recorded inverse Simpson index for all helminths was for a samples of *S. frederici* ($n = 160$), and this species had the second highest mean alpha diversity per sample (10.5 ± 2.9). *Hymenolepis diminuta* alpha diversity was significantly lower than for any other helminth species (1.9 ± 0.3; d.f. = 273, $Z = -2.14$, $p = 0.03$).

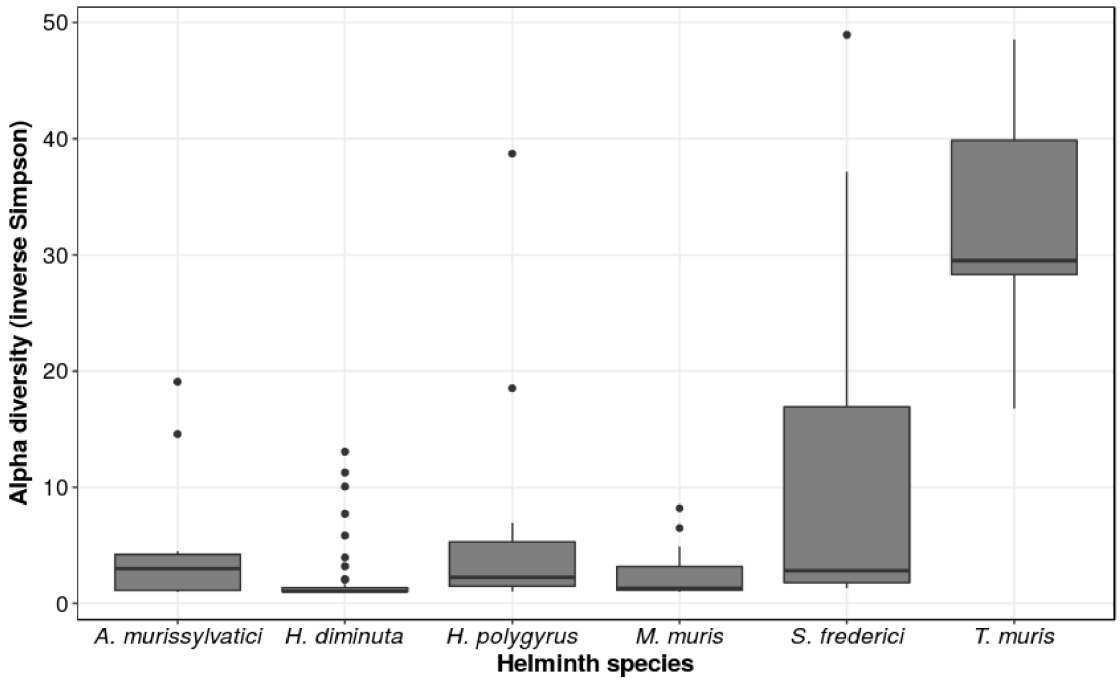

Figure 5.4: Inverse Simpson index of alpha diversity of microbiota from six helminth species isolated from the guts of 32 *Apodemus flavicollis*. Boxes demonstrate the upper and lower quartiles, with median alpha diversity indicated. Bars represent the minimum and maximum range of alpha diversity.

5.4.2 Intraspecific variation of helminth microbiota between gut locations

Both *H. polygyrus* and *T. muris* were found in one gut location, however the other four helminth species were found across multiple gut locations. Intraspecific variation in taxonomic

composition of microbiota was associated with gut location; the taxonomic composition of *A. murissylvatici* microbiota significantly differed between samples isolated from the stomach, small intestine and caecum (Bray-Curtis: d.f. = 8, $F = 1.70$, $p = 0.02$; weighted UniFrac: d.f. = 8, $F = 2.72$, $p = 0.02$). However, it should be noted that only one *A. murissylvatici* was isolated from the caecum, and one from the small intestine. The majority of *M. muris* (88.9%) were mainly isolated from the stomach, but were also present in the small intestine (8.3%) and distal colon (2.8%), and taxonomic composition significantly varied between samples from each of these locations (Bray-Curtis: d.f. = 33, $F = 2.60$, $p = 0.04$; weighted UniFrac: d.f. = 33, $F = 3.36$, $p = 0.02$). Microbiota of *S. frederici* that were found in the small intestine, caecum and proximal colon were also significantly different to one another (Bray-Curtis: d.f. = 19, $F = 2.41$, $p = 0.01$; weighted UniFrac: d.f. = 19, $F = 2.36$, $p = 0.01$). However *H. diminuta* microbiota was not significantly associated with gut location (Bray-Curtis: d.f. = 61, $F = 0.99$, $p = 0.43$; weighted UniFrac: d.f. = 61, $F = 1.83$, $p = 0.14$).

5.4.3 Comparison of helminth and gut microbiota diversity

Across both helminth and gut samples, 354 different bacterial genera were identified. Of these, 189 occurred in both gut and helminth samples, and 16 were found uniquely within helminths, with the remaining 149 present only in gut samples. In general, alpha diversity (as measured by inverse Simpson index) of each helminth species was lower than that of the gut location from within which it was isolated (Figure 5.5). However, five out of six of the helminth species were associated with a microbiota with higher alpha diversity than that of the gut microbiota in at least one sample. Two *A. murissylvatici* samples possessed a microbiota of greater alpha diversity than stomach microbiota, but overall stomach alpha diversity was significantly higher (d.f. = 33, $W = 190$, $p = 0.01$; Figure 5.5). Likewise, in the small intestine four samples of *H. polygyrus*, two of *H. diminuta*, and one of *S. frederici* had microbiota with higher alpha diversity than the respective small intestine microbiota, but small intestine microbiota mean alpha diversity still

remained higher than for helminths (d.f. = 31, $W = 530$, $p < 0.01$; d.f. = 40, $W = 1{,}800$, $p < 0.01$; d.f. = 1, $W = 32$, $p = 1$ respectively.) In addition, four *T. muris* samples from the caeca were associated with higher microbial diversity than the caecum in which they were present (d.f. = 12, $W = 62$, $p = 0.5$; Figure 5.5).

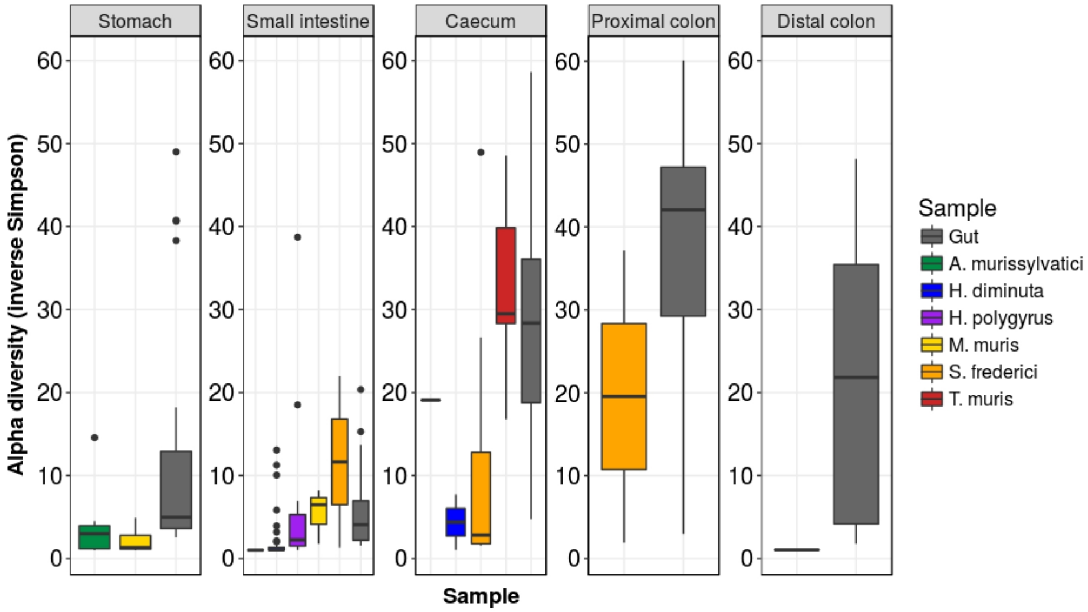

Figure 5.5: Inverse Simpson index of alpha diversity of microbiota associated with different gut locations, as well as alpha diversity of microbiota associated with six helminths species isolated from each respective gut location. Boxes demonstrate the upper and lower quartiles, with median alpha diversity indicated. Bars represent the minimum and maximum range of alpha diversity.

5.4.4 Comparison of helminth and gut microbiota composition

The taxonomic composition of helminth-associated microbiota was compared with that of the respective gut section in which the helminth was found within. *Aonchotheca murissylvatici* and *M. muris*, both found in the stomach, harboured a significantly different microbiota composition to this gut section (Bray-Curtis: d.f. = 64, $F = 9.09$, $p<0.01$; weighted UniFrac: d.f. = 64, $F = 7.54$, $p<0.01$; Figure 5.6). Microbiota of both *H. diminuta* and *H. polygyrus* ordinated away from small intestine microbiota, whereas *S. frederici* and *M. muris* microbiota ordinated more closely with the microbiota of this gut section (Figure 5.6). Regardless, microbial composition of helminths found in the small intestine differed significantly from small intestine microbiota

(Bray-Curtis: d.f. = 112, $F = 3.95$, $p<0.01$; weighted UniFrac: d.f. = 112, $F = 6.21$, $p <0.01$). In addition, microbiota of helminth samples isolated from the caecum significantly differed to caecum microbiota (Bray-Curtis: d.f. = 45, $F = 3.83$, $p<0.01$; weighted UniFrac: d.f. = 45, $F = 5.8$, $p<0.01$).

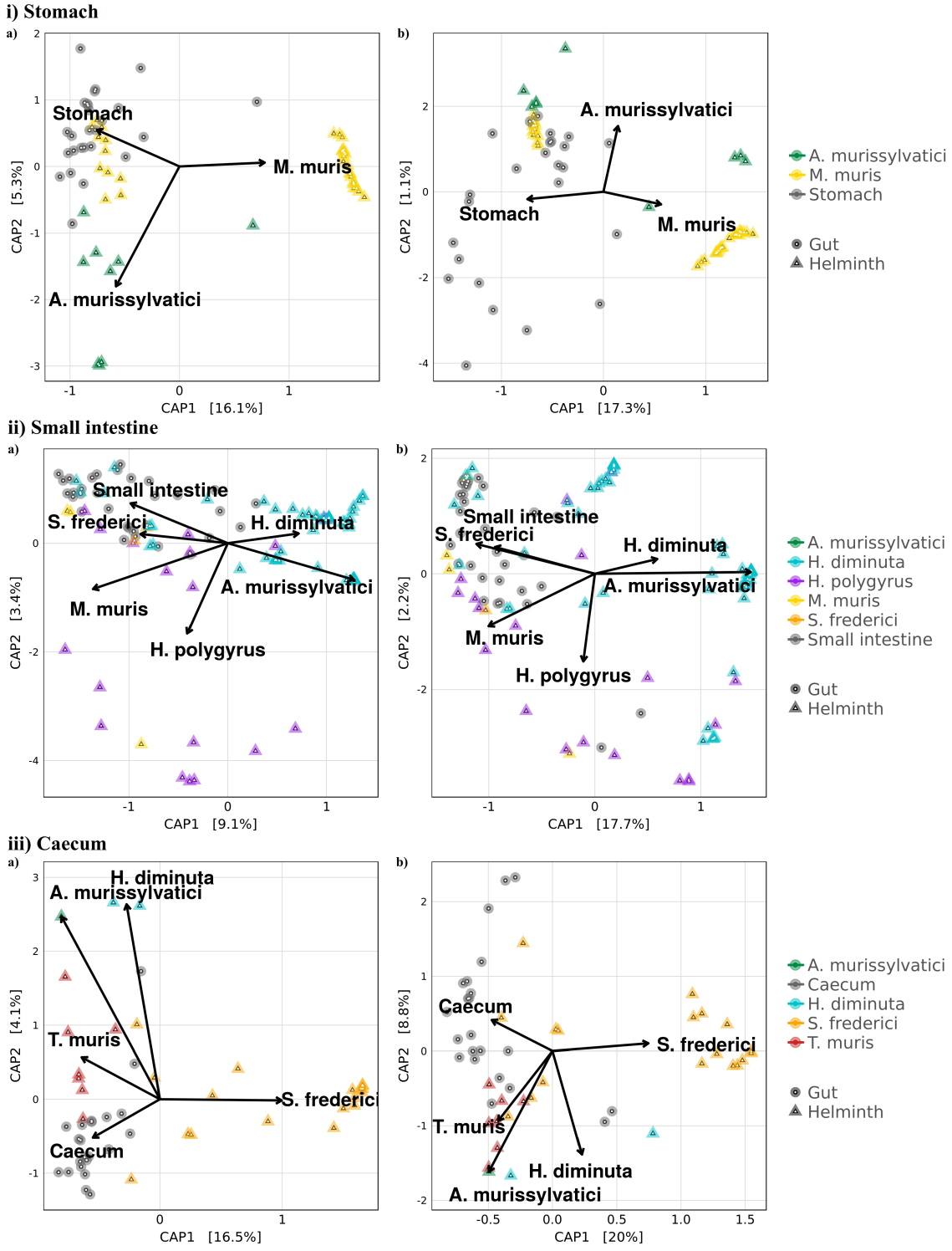

Figure 5.6: Ordination plots of divergence of microbiota taxonomic composition between the i) stomach, ii) small intestine and iii) caecum and of the helminth species therein based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation are shown.

5.4.5 Variation in OTU abundances between helminth and gut microbiota

OTUs from 8 phyla (14 classes) were present in significantly different abundances between the

stomach and the helminths therein (Figure 5.7), the largest range compared to helminths in any other gut section. *Aonchotheca murissylvatici* and *M. muris* microbiota showed similar patterns in bacterial classes that were significantly different in abundance compared to the stomach microbiota. For example, OTUs from the *Proteobacteria* phylum were significantly higher in abundance in the microbiota of both of these helminth species compared to stomach microbiota, whilst OTUs from 11 common classes were lower in abundance (Figure 5.7; see Appendix A.6, Table A.6.1 and A.6.2 for detailed statistics from these DESeq analyses).

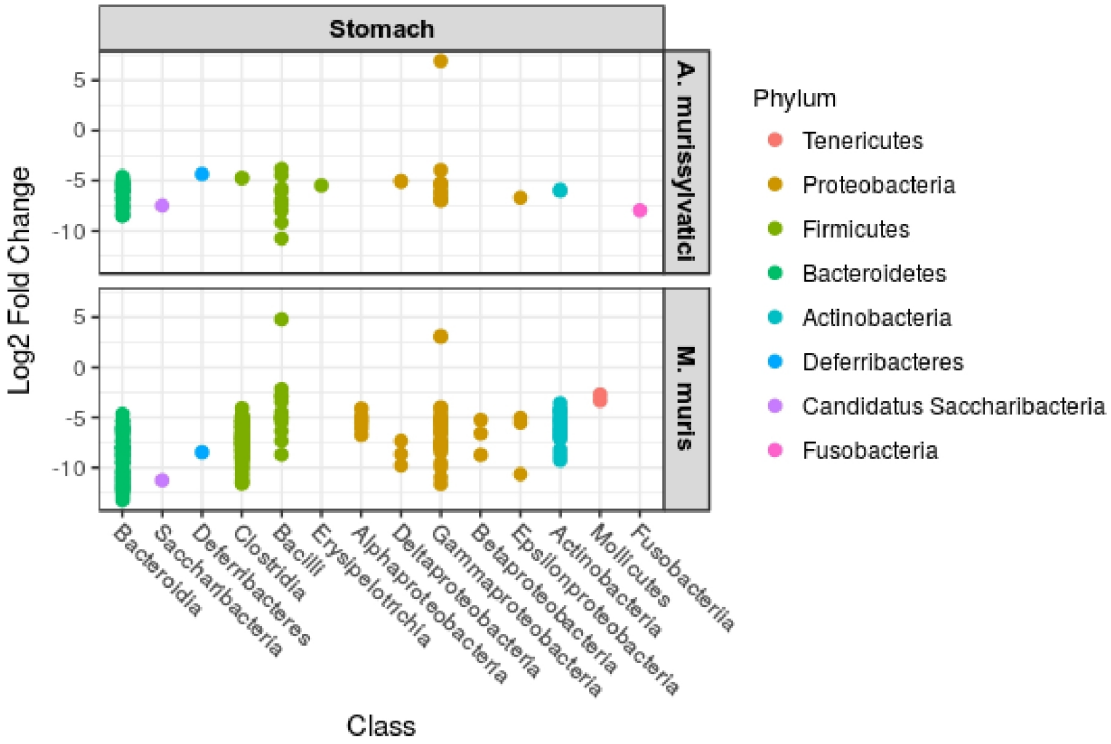

Figure 5.7: OTUs in the microbiota of helminths isolated from the stomach (*Aonchotheca murissylvatici* and *Mastophorus muris*) that were significantly different to those present in the stomach microbiota. OTUs were grouped by microbial class. Briefly, DESeq was used to identify significantly changing (p<0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing helminth to stomach microbiota.

Mollicutes was consistently present in significantly higher abundances in helminths isolated form the small intestine, compared to microbiota of the small intestine itself (Figure 5.8). OTUs from the phylum *Proteobacteria* were also in significantly higher abundances in helminth-associated compared to small intestine microbiota. In addition, OTUs from 12 classes (7 phyla) were lower in abundance in helminth compared to small intestine microbiota (Figure 5.8; see Appendix A.6,

Table A.6.3, A.6.4 and A.6.5 for detailed statistics from these DESeq analyses).

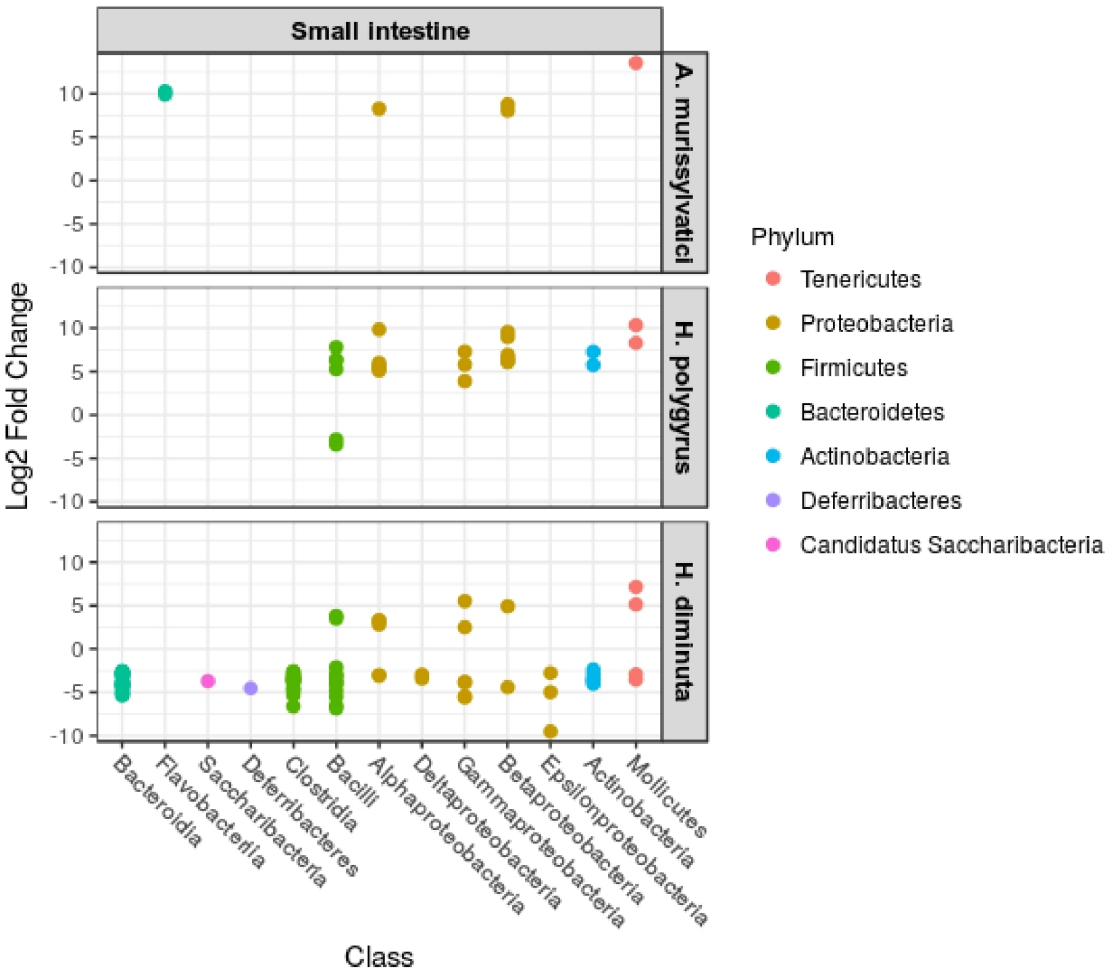

Figure 5.8: OTUs in the microbiota of helminths isolated from the small intestine (*Aonchotheca murissylvatici, Heligmosomoides polygyrus* and *Hymenolepis diminuta*) that were significantly different to those present in the small intestine microbiota. OTUs were grouped by microbial class. Briefly, DESeq was used to identify significantly changing (p<0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing helminth to small intestine microbiota.

In the four helminth species isolated from the caecum, OTUs from 10 classes (6 phyla) were significantly higher in abundance, and OTUs from 10 classes (7 phyla) were significantly lower in abundance compared to in caecum microbiota (Figure 5.9). Notably, OTUs from the classes *Bacteroidia*, *Clostridia* and *Bacilli* were persistently present in abundances that significantly differed to those in the caecum microbiota (Figure 5.9; see Appendix A.6, Table A.6.6, A.6.7, A.6.8 and A.6.9 for detailed statistics from these DESeq analyses).

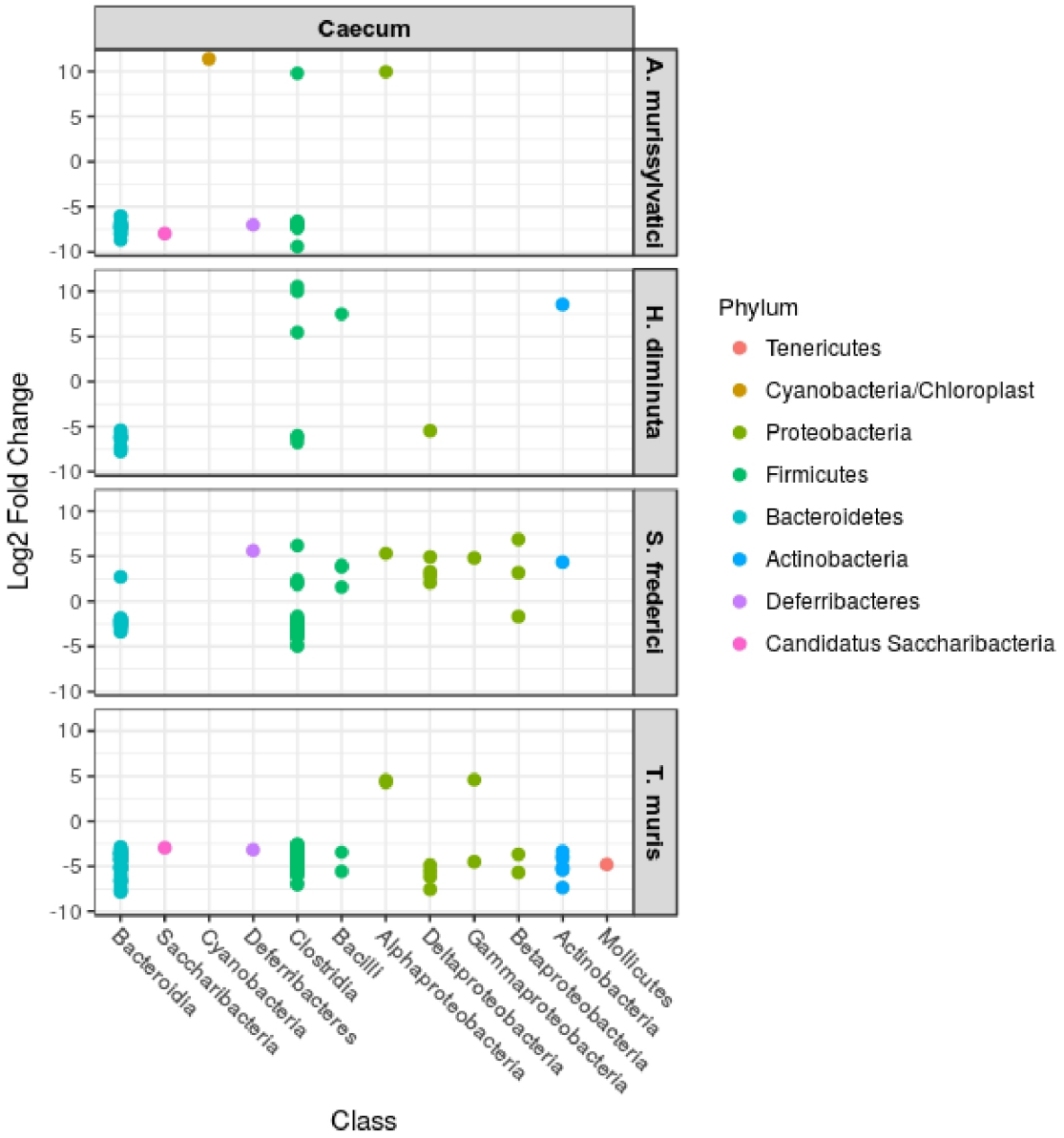

Figure 5.9: OTUs in the microbiota of helminths isolated from the caecum (*Aonchotheca murissylvatici, Hymenolepis diminuta, Syphacia frederici* and *Trichuris muris*) that were significantly different to those present in the caecum microbiota. OTUs were grouped by microbial class. Briefly, DESeq was used to identify significantly changing (p<0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing helminth to caecum microbiota.

Only two samples of *Syphacia* were found within proximal colon samples, and OTUs from two classes of bacteria associated with these helminths significantly differed in abundance compared to proximal colon microbiota; *Gammaproteobacteria* and *Actinobacteria* (see Appendix A.6, Table A.6.10 for detailed statistics from these DESeq analyses). In the distal colon, a single *M. muris* possessed a monoculture microbiota of *Gammaproteobacteria* (Figure 5.10). Indeed,

Gammaproteobacteria was more than 11 $\log^2$ fold higher in the helminth microbiota compared to the distal colon (see Appendix A.6, Table A.6.11 for detailed statistics from these DESeq analyses).

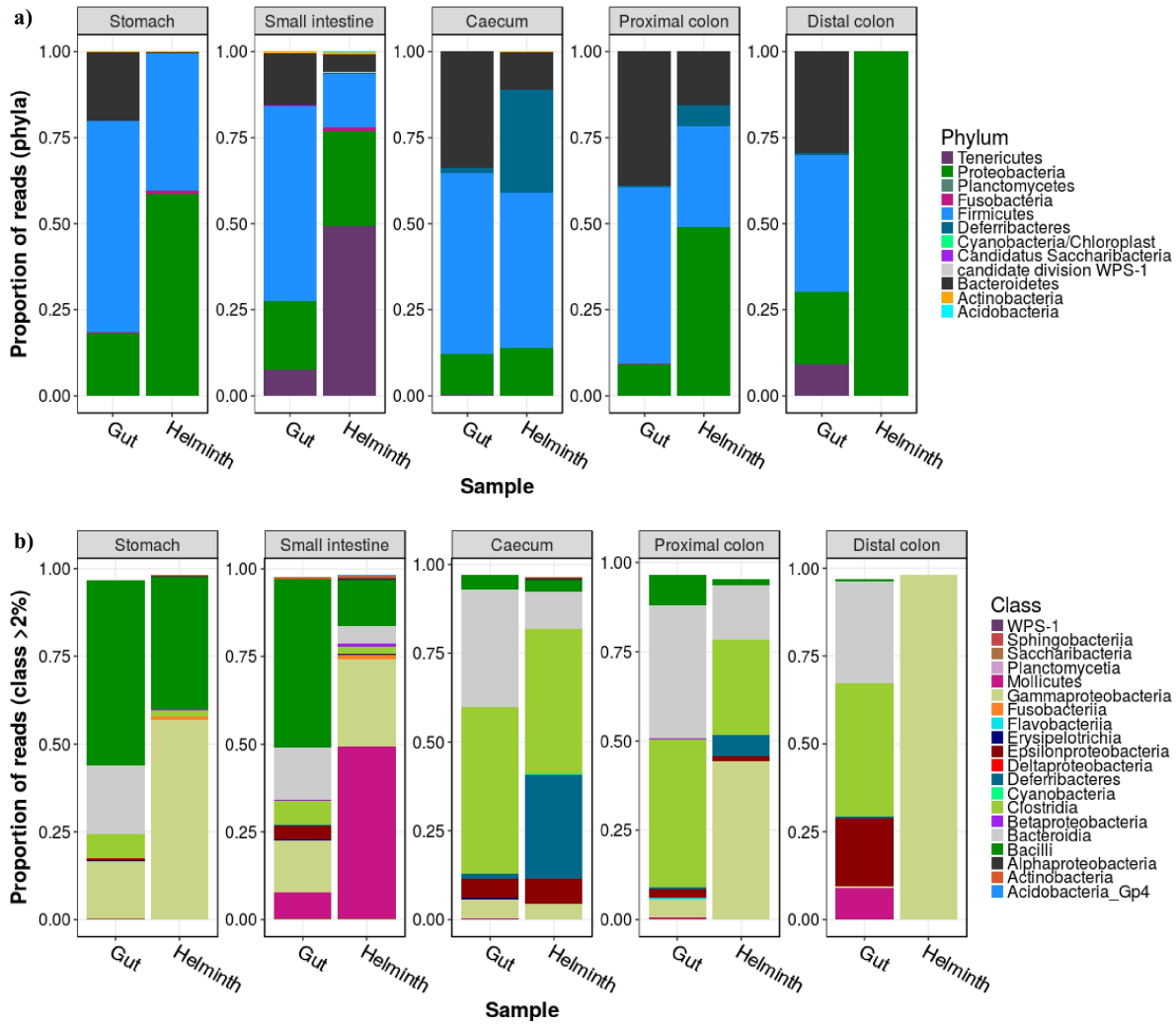

Figure 5.10: Proportion of reads of bacterial a) phyla and b) classes (composing >2% reads) for gut and all helminth samples located within for five gut locations. Gut and helminth samples were collected from 32 *Apodemus flavicollis*.

5.5 Discussion

Here, composition and diversity of microbiota associated with parasitic helminths was quantified for five nematode species and one cestode species, all of which were isolated from the gastrointestinal tracts of naturally infected, wild rodents. Each species of helminth was associated with a unique microbiota, and exhibited intraspecific diversity which was significantly

associated with inhabitation of different gut sections. In addition, helminth-associated microbiota was significantly different to gut microbiota.

Although microbiota composition of each helminth species was dominated (>10% of mean reads) by *Firmicutes, Proteobacteria* and *Tenericutes*, the relative abundances of these phyla varied, such that each species arguably had a distinct microbiota (Figure 5.2 and 5.3). Of note, the microbiota of *S. frederici* was unique compared to that of other helminth species because *Deferribacteres* was also a dominant bacteria and constituted more than 1/3 of the microbiota community. In addition, helminth microbiota exhibited intraspecific variation in diversity and composition (Figure 5.1, 5.2 and 5.3). Interestingly, more than half of the sequenced samples of *H. diminuta* were associated with a monoculture microbiota, including *Mycoplasma, Escherichia/Shigella* and *Lactobacillus.* Likewise, *A. murissylvatici* samples had a monoculture microbiota of *Mycoplasma,* and a single *M. muris* had a microbiota composd of monoculture of *Escherichia/Shigella.* Members of both the *Escherichia/Shigella* and *Mycoplasma* genera are pathogenic to vertebrates; some species of *Shigella* can cause bacillary dysentery, and invade the epithelia of the colon and rectum, eventually leading to severe tissue damage. Similarly, members of the *Shigella* genus (*S. flexneri*) invade the intestinal cells of, and even kill the *Caenorhabditis elegans* nematode (Burton *et al.*, 2006; Kesika *et al.*, 2011; George *et al.*, 2014), however it is unknown if these bacteria are also pathogenic to the helminth species presented here, and further analyses including culture dependent techniques would be necessary to confirm that the OTUs observed here were indeed pathogenic species.

All but two helminth species (*H. polygyrus* and *T. muris*) were present in multiple sections of the gut, and taxonomic composition and diversity of microbiota significantly differed between samples isolated from different gut locations. However, the microbiota associated with the cestode species, *H. diminuta*, did not significantly differ between gut locations. Although *A.*

murissylvatici significantly clustered between the stomach, small intestine and caecum, little can be robustly ascertained from this result, as only one helminth individual was found from the small intestine and one from the caecum. Interestingly, *M. muris* were found in the stomach, small intestine and distal colon, and differences in helminth microbiota were significantly associated with gut location. Normally, *M. muris* infect the stomach (Lafferty *et al.*, 2010; Grzybek *et al.*, 2015), thus it was unusual to find this species in the small intestine or distal colon; it is possible that these individuals were in the process of being ejected by the host. As such, it could be speculated that the microbiota of *M. muris* from these gut sections varied because the helminth had died, and/or an immune response from the host that had acted upon the helminth to stimulate ejection had also altered the helminth microbiota composition.

In general, helminth microbiota had lower alpha diversity than the microbiota of the gut section from within which it was isolated (Figure 5.5). It is no surprise that the bacteria able to flourish within the gut may not also colonise the helminth, as some bacteria species have very specific growth requirements (as demonstrated by the limited success of culture-dependent techniques e.g., Suau *et al.*, 1999), which the helminth may not provide. Conversely, much like in the vertebrate gut (Rawls *et al.*, 2006), assembly of the helminth microbiota is not random (Berg *et al.*, 2016), and may be controlled to allow or prevent the growth of bacterial species that are beneficial (or not) to the helminth host. However, with the exception of *M. muris,* all helminth species possessed a microbiota with higher alpha diversity than the respective gut section in which it was found on at least one occasion, suggesting that microbiota were acquired from sources other than the gut location (Figure 5.5).

Of the 189 bacterial genera identified across all helminth and gut samples, 16 occurred exclusively in helminths, suggesting that bacteria may be acquired from other sources in addition to the definitive host. Many helminth species have a free-living stage outside of the host and may

undergo development in the environment or an intermediate host, during which time the helminth could be colonised by microbes. For example, *H. diminuta* have an indirect life-cycle; eggs are ingested by an insect intermediate host, penetrate the gut, and develop in the haemocoel. The definitive host (small mammal) becomes infected when it eats an insect infected with *H. diminuta* cysticercoids (infective stage, Smyth, 1994). It is therefore possible that *H. diminuta* possess microbiota which originates from the insect intermediate host, either through ingestion of microbes while in the intermediate host, or by colonisation of microbiota on the exterior of the helminth. In another example, the eggs of *H. polygyrus* are shed in host faeces and hatch in the environment. Following a moult, the L2 larvae feed on bacteria within the environment, and partially moult again into ensheathed, L3 infective larvae, which are non-feeding. The larvae become ex-sheathed following ingestion by a host (Bryant, 1973; Valanparambil *et al.*, 2014). Although larvae are non-feeding once they become infective, until after they develop into tissue-feeding adults in the gut, they may still harbour microbes from during the bacteria-feeding L2 stage, which would be acquired from host faeces and the environment. *Syphacia frederici* are unlikely to have acquired microbiota in the environment in the same way, as the life-cycle of this genus is direct and may involve retroinfection (Prince, 1950). However, helminths are mobile within the gut and could be colonised by microbiota from multiple gut locations; both *H. diminuta* and *S. frederici* are reported to have a circadian routine of migration in the gut, e.g., *S. frederici* migrate from the caecum to the rectum to lay eggs (Kerboeuf and Lewis, 1987). Adult *S. frederici* typically inhabit the caecum, suggesting that helminths may have acquired microbiota from the microbially richer habitat of the caecum and distal gut sections, and later been displaced into the small intestine.

Helminths were rinsed with TBS to remove external debris associated with the host gut, and it is highly likely that microbes primarily associated with the host gut remained on the exterior of the helminth. Despite the high chance that these external host microbes were a significant

contribution to helminth-associated microbiota, this was not the case. Indeed, in the majority of instances helminth microbiota clustered away from the microbiota of the gut section within which they were isolated (Figure 5.6). The disparity between the taxonomic compositions of the gut and helminth microbiota provides further evidence that helminths either acquire microbiota from additional sources other than the host gut (e.g., the environment or an intermediate host) and/or helminths do not passively obtain microbes, but microbiota acquisition and composition is structured according to the needs of the helminth (Berg *et al.*, 2016).

The current study provides the first account of interspecific and intraspecific variation in microbiota of a whole community of helminths, and dissimilarities between the associated gut microbiota. Previous studies have characterised the microbiota of endoparasites; the liver fluke *Opisthorchis viverrini* (Plieskatt *et al.*, 2013) and *H. polygyrus* (Walk *et al.*, 2010). As suggested here, Plieskatt *et al.,* (2013) proposed that the liver fluke is capable of relocating microbiota during its migration through the host body. The sequences obtained here for *H. polygyrus* are rather different to those reported by Walk *et al.,* (2010); here 14 bacterial families were identified, whereas Walk *et al.,* (2010) identified nine families, and only two of these families are common between the two studies (*Lactobacilliaceae* and *Erysipelotrichaceae*). In addition, in the laboratory study, *Lactobacillaceae* dominated the *H. polygyrus* microbiota in more than 50% of reads, whereas in the current study it only constituted 21.8% of the microbiota, and instead *Mycoplasma* were the dominant family (40.7%; Walk *et al.*, 2010). The disparity in the current results compared to those of Walk *et al.,* (2010) are likely due to the fact that in the present study helminths were isolated from naturally infected wild animals, as opposed to helminths that had been artificially cultured and administered as an experimental infection to laboratory rodents, which would likely result in helminths with an altered and depauperate microbiota due to a lack of environmental acquisition. It is also important to note that Walk *et al.,* (2010) utilised a different method of bacterial DNA sequencing (Sanger-style and quantitative PCR), which may

have effected results.

To summarise, parasitic helminths are associated with a microbiota, which shows intraspecific variation associated with inhabitation of different gut section. Helminth microbiota is largely composed of the common gut phyla *Firmicutes* and *Proteobacteria,* as well as *Tenericutes*, and diversity of microbiota is generally lower than of the gut. However, in some instances helminth microbiota diversity exceeds that of the gut, and shows significant differences in taxonomic composition and OTU abundances, suggesting that helminths may acquire microbiota from prior life stages e.g., from the environment or an intermediate host, and/or the helminth allows selective colonisation of microbes (Berg *et al.*, 2016). The data presented here provide the first step towards addressing the potential use of antibiotics to treat helminth infection, as initially tested more than 60 years ago (Wells, 1951, 1952a, 1952b; Brown, 1952; Chan, 1952; Salem and el-Allaf, 1969; Hoerauf *et al.*, 1999; Saint André *et al.*, 2002). Further research to identify bacteria that are key symbionts of helminths, perhaps by identifying how and where helminths acquire microbiota could indicate specific targets for removal as a form of helminth control. Antibiotic treatment following helminth infection may also mitigate the introduction of microbes by the helminth that are potentially pathogenic to the host such as those identified here; e.g., *Escherichia/Shigella.*

5.6 Author Acknowledgements

The manuscript resulting from this chapter is authored by:

Emily L. Pascoe, Francesca Albonico (Fondazione Edmund Mach), Matthew J. Bull (Cardiff University), Heidi C. Hauffe (Fondazione Edmund Mach) and Sarah E. Perkins (Cardiff University and Fondazione Edmund Mach).

Chapter 6

Faecal microbiota affects helminth development

“I love fools' experiments. I am always making them”

Charles R. Darwin

6.1 Abstract

Gut microbiota is integral to immunity, and differing microbial compositions between individuals have been linked to specific immune phenotypes that provide defence against pathogens. Immune responses linked to gut microbiota composition have been observed in response to adult helminths inhabiting the gut, including phenotypes that can impact upon helminth development. However, the eggs of many helminth species are expelled in host faeces, and may subsequently undergo development and hatch in faecal microbiota. The current study investigates if faecal microbiota could be an extended immune phenotype of the host by also affecting helminth development. Differences in probability and rate of egg development between eggs cultured in different faecal microbiota were measured. Transplants of eggs into 'self' faeces, and faeces of a randomly selected 'non-self' individual, were performed for eggs of *Heligmosomoides polygyrus* and *Trichuris muris* isolated from the faeces of naturally infected wild mice, *Apodemus flavicollis*. On average, significantly more *H. polygyrus* eggs ($Z = 2.32$, $p = 0.02$) hatched in non-self (40.3%) compared to self faeces (20.4%). Probability of hatching was not significantly associated with the alpha diversity of self or non-self faecal microbiota, nor with the faecal egg burden of self or non-self faeces. There was no significant difference in rate of *H. polygyrus* egg hatching between self and non-self faeces ($p = 0.41$). In contrast, the probability of *T. muris* egg development was lower in non-self (94.4%, ±3.93% standard error), compared to self faeces (100%; ±0%), but this difference was not significant ($p = 0.30$). The results suggest that faeces provide an extended immune phenotype of the host, and can reduce the probability and rate of egg hatching of some helminth species, ultimately reducing the development of this parasite.

6.2 Introduction

Gut microbiota is vital for immune system development and function; the human foetus, which has been argued to develop in an almost sterile environment, and germ-free mice both exhibit immature immune systems, which develop following colonisation of the gut by bacteria (Round and Mazmanian, 2009; Weng and Walker, 2013). Microbiota stimulates the function and development of immune cells, as well as pro-inflammatory responses, so that the host may be primed to defend against pathogen invasion (Cahenzli *et al.*, 2012; Chung *et al.*, 2012; Wingender *et al.*, 2012; Buffie and Pamer, 2013). In turn, the microbiota can lead the host to express distinct immune phenotypes, for example, the first source of bacterial inoculum received by humans can influence susceptibility to autoimmune diseases; babies delivered by caesarean section are initially colonised by skin microbes as opposed to vaginally born individuals that receive an inoculum of faecal and vaginal microbes (Dominguez-Bello *et al.*, 2010; Neu and Rushing, 2011; Jakobsson *et al.*, 2014). As a result of this difference in gut colonisation, the development of the immune system differs between caesarean section and vaginally born babies, such that caesarean section babies are more predisposed than those vaginally born to asthma and other autoimmune diseases (Jakobsson *et al.*, 2014). Specific bacterial compositions have also been linked to increased susceptibility to inflammatory bowel diseases (Hold *et al.*, 2014), viral replication (Kuss *et al.*, 2011), and resistance to pathogenic bacteria such as *Salmonella* spp. (Bäumler and Sperandio, 2016). Immune phenotypes associated with gut microbiota are, like the microbe composition, highly dynamic, and can change when the microbiota is altered by antibiotic or probiotic treatment (e.g., Bautista-Garfias *et al.*, 2001; Martínez-Gómez *et al.*, 2009; Kuss *et al.*, 2011; Weng and Walker, 2013). In addition, microbiota has been attributed as a stronger driver of specific immune defences than genotype, and the immune phenotype can be transferred between individuals by microbiota transplant (Koch and Schmid-Hempel, 2012), and vertically

transmission (Oliver *et al.*, 2014).

Due to the shared evolutionary history of microbiota and parasitic helminths within the gut, as well as microbiota-immunity interplay, it is not surprising that bacteria in the gut can provide the host with resistance (the ability of a host to reduce establishment) to macroparasites (e.g., Hayes *et al.*, 2010; Coêlho *et al.*, 2013; Reynolds *et al.*, 2014). Parasites are in a constant arms race with their host to evolve adaptations so that each maintains their relative fitness (Brockhurst *et al.*, 2014). For example, the host may mount an immune response against a given parasite, which the parasite in turn can override (Maizels *et al.*, 2004). The response by the host to a helminth infection may target any given life stage of the parasite. For instance, particular bacterial families have been associated with host immunity against adult helminth fecundity and/or abundance. Administration of probiotics which increase the abundance of *Lactobacillus* bacteria in the gut can result in an anthelmintic effect in domestic dogs, leading to a decrease in the number of hookworm eggs (from the Ancylostomatidae family) shed in faeces (Coêlho *et al.*, 2013) and can promote an immune response in mice against *Trichinella spiralis*, causing a decrease in the number of adults and larvae in the gut (Bautista-Garfias *et al.*, 2001; Martínez-Gómez *et al.*, 2009). However, when *T. spiralis* are cultured *in vitro* in the presence of *Lactobacillus* there is a positive effect on the number of adult helminths able to survive, and their subsequent fecundity (Jiang *et al.*, 2016), suggesting that other bacteria within the gut may contribute to the anthelmintic effects of *Lactobacillus* observed *in vivo.*

Gut bacteria have also been associated with immune phenotypes related to helminth development and egg hatching, e.g., larvae of the laboratory rodent nematode, *Heligmosomoides polygyrus* (*bakeri*) reared in axenic conditions do not survive past the L2 stage as the body wall develops

with malformations (Weinstein *et al.*, 1969). In addition, the eggs of *Trichuris muris,* which hatch within the mouse gut, require physical contact with specific bacteria, e.g., *Enterococcus caccae, Staphylococcus aureus* and *Streptococcus hyointestinalis,* and other common gut bacteria with type 1 fimbriae, such as *Escherichia coli* and *Salmonella typhimurium* to activate the hatching process (Hayes *et al.*, 2010; Koyama, 2013; Vejzagić *et al.*, 2015a, 2015b). Simultaneously, other physical conditions of the gut previously believed to provide a hatching cue, such as low pH or gastric enzymes, are unnecessary for *T. muris* hatching (Hayes *et al.*, 2010; Wimmersberger *et al.*, 2013). The life-cycle of faecal-oral transmitted parasites is such that progeny are expelled in the faeces, in which they typically hatch and develop into the infective stage of the life-cycle, before being able to infect a host. Whilst in the faeces, helminth eggs are in direct contact with the unique faecal microbiota the host. Given that gut microbiota are associated with resistance to helminth infection, it is not unreasonable to assume that faecal microbiota, which originates from the gut, could also provide some helminth resistance.

In addition to the direct impacts of bacteria on helminth development, microbiota may affect the host immune responses to parasites (Koch and Schmid-Hempel, 2012; Weng and Walker, 2013), the composition of which can change following helminth infection (Walk *et al.*, 2010; Li *et al.*, 2012; Rausch *et al.*, 2013; Reynolds *et al.*, 2014), with potential subsequent effects on immune phenotype. For instance, faeces of infected individuals may provide the host with resistance to helminth infection; the host can produce antibodies against the helminth which are shed in faeces, binding to the egg and inhibiting development (Jørgensen *et al.*, 1998), a response that could be modulated by microbiota (Reynolds *et al.*, 2015). However, the eggs of some nematode species are unaffected by antibodies present in faeces (Lambert *et al.*, 2015).

Understanding if, and how, faecal microbiota affects helminth development will not only alter current perceptions of the host immune phenotype and the ability of helminths to develop in faeces, but could also have implications for human and livestock health, as the ability to disrupt parasite development can be an effective method of its control and eradication (e.g., Barry, 2007). The development of eggs from two helminth species, *H. polygyrus* and *T. muris,* which are both shed and undergo development in host faeces*,* were tested in faeces from different individuals of a naturally infected wild rodent (yellow-necked mouse; *Apodemus flavicollis*). Following sterilisation of external egg-associated microbiota, eggs were cultured in faeces from the host in which they were shed ('self') and in faeces with a different microbiota diversity and composition from another randomly selected individual ('non-self'), and the probability and rate of egg development between culture in self and non-self faeces were compared. In addition, helminth egg burden of faeces (prior to the removal of eggs for preparation as a faecal microbiota medium) and microbiota diversity were tested as possible impacting factors on helminth egg development.

6.3 Materials and methods

6.3.1 Study area and rodent sampling

Live-trapping of *A. flavicollis* was conducted using Ugglan multi-capture live traps (Ugglan Type 2; Grahnab, Sweden) arranged in two transects of 100 traps each, with a 10 m inter-trap interval. Transects, which were separated by 500 m of vineyard, were situated in San Michele all'Adige, Trento (transects situated at 46°11'31.6"N 11°08'20.2"E and 46°11'17.9"N 11°08'16.2"E). Traps were baited with sunflower seeds and potato between March and June, for four nights per week, during which time they were checked every 24 hours. Animal trapping and handling procedures were authorised by the Comitato Faunistico Provinciale della Provincia di Trento, prot. n. 595

issued on 04 May 2011.

At first capture of each mouse, sex was recorded (known to influence the helminth community and microbiota of mice; Ferrari *et al.*, 2004; Markle *et al.*, 2013) and a Passive Integrated Transponder tag (Trovan™ ID 100; Trovan Ltd., UK) was inserted subcutaneously to identify the individual at subsequent capture events. Faeces were collected from each trap occupied by a single animal. During each trapping week, faeces collected at first weekly capture of an individual were frozen at -80°C for future faecal microbiota analyses (see *'6.3.5 Microbiota analysis'* below). Faeces from subsequent recaptures of an individual during that week were used for faecal egg count (FEC) analyses, using a standard McMaster technique with saturated NaCl flotation solution (after Dunn and Keymer, 1986). The mean number of eggs per gram (EPG) of faeces (including zeros) was calculated from all FEC measurements collected for a given individual to account for daily variation in egg shedding (Michael and Bundy, 1989; Kumazawa, 1992). After occupation, traps were sterilised using sodium hypochlorite (bleach), followed by 4% chlorhexidine solution (Nuova Farmec, Italy), re-baited and replaced. Each mouse (with the exception of pregnant/nursing females, juveniles and individuals trapped <4 days previously) was then transferred into a sterilised Longworth trap (Longworth Scientific Instruments Co., United Kingdom) containing sunflower seeds, potato and hay, and kept overnight *in situ*. The following morning mice were released from Longworth traps, and faecal samples within were transported to the laboratory at 4°C for use in an egg transplant (see '*6.3.3 Egg transplant: culture in 'self' and 'non-self' faeces'* below). In the laboratory, each faecal sample was immediately placed on filter paper (previously sterilised under UV light) which was saturated with ultra-pure water in a sealed Petri dish at 4°C, for 2 hours to standardise humidity content.

6.3.2 Egg isolation and sterilisation of external microbiota

Each faecal sample from animals kept overnight was termed an egg 'recipient' and was processed in the following way. Faeces were homogenised with sterile Tris-NaCl buffered saline (TBS: 50 mM Tris, 200 mM NaCl pH 8) at a ratio of 1 g/10 ml, and centrifuged at 700 G for 3 minutes. The resulting supernatant containing bacteria (henceforth referred to as 'faecal bacteria solution') was maintained at 4°C until further use. Meanwhile, the pellet containing eggs and faecal debris was re-suspended in TBS and passed through a 1 mm strainer to remove larger faecal debris, followed by three cell strainers (pluriSelect® pluriStrainers, Germany) of decreasing pore size (200 µm, 100 µm and 40 µm) to progressively remove smaller debris, whilst capturing helminth eggs. As most bacteria are 0.2 - 2.0 µm in diameter (Tortora *et al.*, 2009), faecal microbiota could pass through all filters. The liquid filtrate was collected and pooled with the previously prepared faecal bacteria solution, and subsequently passed through a 15 µm pore strainer to ensure it was free of all eggs (this step was found to be necessary during a pilot experiment in which a FEC was performed on aliquots of faecal bacteria solution to ensure it was egg-free). Eggs of *H. polygyrus* are typically 75.0 ± 5.5 µm × 49.2 ± 3.1 µm; (Camberis *et al.*, 2003) and *T. muris* eggs are <74.5 µm long (Koyama, 2013), thus eggs were captured on the 40 µm and 15 µm strainers, where they were retained throughout the following external sterilisation procedure. Strainers containing eggs were washed with 15 ml of TBS, submerged in 15 ml of 4% chlorhexidine solution for 5 minutes and rinsed with a further 15 ml of TBS. In a pilot experiment, there was no visible growth of bacteria resulting from sterilised eggs after five days of culture on OP50 substrate, and egg viability was unaffected (data not shown). Sterilised eggs were transferred into a Petri dish by inverting the strainer and washing through with TBS, and eggs were subsequently separated according to species (*H. polygyrus* or *T. muris,* other species were discarded due to difficulties in culturing *in vitro*), and maintained in TBS during preparation of culture dishes (see '*6.3.3 Egg*

transplant'). Individuals whose faeces contained eggs were also designated as an egg 'donor' to donate eggs to either 'self' or 'non-self' faeces of egg recipients (see '*6.3.3 Egg transplant*' and Appendix A.7, Table A.7.1). However, due to individual and daily variation in faecal and egg yield, not every individual throughout the study could be designated as both a recipient and a donor (see '*6.3.3 Egg transplant*'). For a full breakdown of usage of faeces from each individual see Appendix A.7, Table A.7.1.

6.3.3 Egg transplant: culture in 'self' and 'non-self' faeces

The following steps were performed under sterile conditions, and each culture dish containing filter paper saturated with ultra-pure water (constructed following methods adapted from Johnston *et al.*, 2015) was sterilised under UV light for 15 minutes immediately prior to the addition of eggs and faeces. The faecal bacteria solution of each recipient was vortexed for 15 seconds and divided into equal aliquots of approximately 5 ml (0.5 g of starting faecal material). Each aliquot equated to a replicate, and was centrifuged at 5500 G for 15 minutes. The resulting supernatant was discarded, and the pellet containing faecal bacteria was spread as a thin 'faecal smear' on filter paper of a culture dish (Figure 6.1). Equal numbers of eggs from each donor were transplanted by pipette into the replicate faecal smears of a recipient to make a 'donor-recipient combination' of individuals; a 'self' combination whereby the donor and recipient were the same individual, and a 'non-self' combination where the recipient was another randomly selected individual (including those from which no eggs were isolated, see Figure 6.1 for schematic representation, see Appendix A.7, Table A.7.1, A.7.2 and A.7.3 for list of egg donor and recipient mice). Due to variation in egg yield between egg donors, the number of eggs per dish varied between different donor-recipient combinations, but remained constant between replicates (range: 1-5 *H. polygyrus* eggs/culture, and 5-10 *T. muris* eggs/culture). For *H. polygyrus,* seven self and

nine non-self donor-recipient combinations were made (from which 16 self and 16 non-self cultures were made, including replicates, see Appendix A.7, Table A.7.2 for details), while for *T. muris* five self and 10 non-self donor-recipient combinations were made (from which 12 self and 13 non-self cultures were made, including replicates, see Appendix A.7, Table A.7.3 for details). To avoid dehydration, 2 ml of ultra-pure water was added to the bottom of each culture dish, which was then sealed with Parafilm® 'M' and maintained at a constant 23°C in the dark.

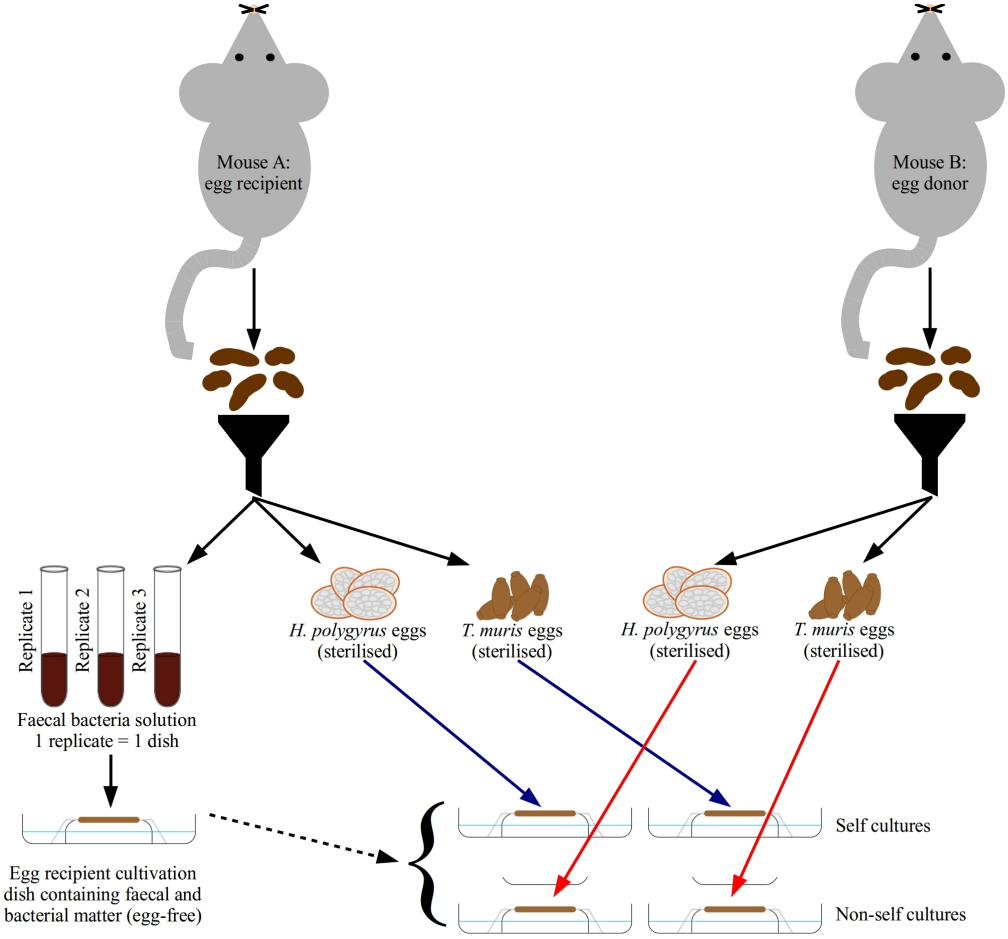

Figure 6.1: Visual representation of experimental design. Each individual or 'egg recipient' was randomly assigned an 'egg donor'. Helminth eggs of *Heligmosomoides polygyrus* and *Trichuris muris* were separated from the faeces of both the egg donor and recipient. Eggs of one helminth species from the recipient ('self culture', blue arrows), and from the donor ('non-self culture', red arrows) were transplanted into the recipient's faeces. The number of replicates of both self and non-self cultures between two individuals varied depending on the faecal yield of each individual and the eggs therein.

6.3.4 Quantification of helminth development

Heligmosomoides polygyrus cultures were checked for hatched larvae daily under sterile conditions at 07:00 and 17:00 for 21 days, as follows. The perimeter of the filter paper of each culture dish was washed with ultra-pure water to dislodge larvae migrating from the faecal smear; this liquid was centrifuged at 1845 G for 5 minutes. The resulting pellet was checked for larvae at 10× magnification. To prevent dehydration, 2 ml of ultra-pure water was added to the culture dish, which was re-sealed with Parafilm® 'M' after every larval check. *Trichuris muris* cultures were checked once, at least six weeks post-culture (range: 6 - 11 weeks; number of weeks had no significant effect on egg degradation, Kendall's Tau: Z = -1, p = 0.3), after embryonation is expected to occur (Zaph and Artis, 2015). For *T. muris*, the faecal smear was scraped from each culture and homogenised with ultra-pure water, before centrifugation at 700 G for 5 minutes. To the resulting pellet, 2 ml of saturated NaCl floatation solution was added to float eggs, and the solution was scanned on a McMaster slide at 100× magnification. The number of embryonated eggs, and eggs that had deteriorated (e.g., shape or structure lost, egg discoloured) were quantified. Hatching and embryonation success were calculated for *H. polygyrus* and *T. muris,* respectively, as a percentage of the total number of eggs in each culture dish.

6.3.5 16S rRNA gene sequencing

For eight individuals, frozen faeces collected for a given individual throughout the experiment (2 – 5 samples from different time points, depending on capture rate of individual) were pooled, to account for any seasonal variation in microbiota throughout the experiment (Maurice *et al.*, 2015). The QIAmp DNA Stool Mini kit (Qiagen, Valencia, CA, USA) was used to extract total genomic DNA from faecal samples. In addition to the methods provided by the manufacturer for pathogen detection, a 2 minute homogenisation step at 30 Hz was performed to enhance bacterial cell lysis,

using a Mixer Mill MM200 (Retsch GmbH, Haan, Germany) with 5 mm stainless steel beads (Qiagen, Valencia, CA, USA). Purity and quality of the recovered DNA were determined using a QIAxcel capillary electrophoresis system (Qiagen, Valencia, CA, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified using the 341F and 805R primers (see Appendix A.2, Figure A.2.1 for details on primer sequences, including degenerate nucleotides), and sequenced using a 2×300 bp kit on the Illumina MiSeq system (Illumina, San Diego, CA, USA). The PCR reactions were carried out in a total volume of 25 µl containing 0.4 µM of each primer, 0.4 mM of dNTP (Promega, Madison, WI, USA), 1× FastStart reaction buffer (Roche Diagnostics GmbH, Mannheim, Germany), 1 mM of $MgCl_2$, 1.25 unit of FastStart HiFi Polymerase (Roche Diagnostics GmbH, Mannheim, Germany), and 12.5 ng of genomic DNA for each sample amplification. Thermal cycling was performed on a GeneAmp™ PCR System 9700 instrument (Thermo Fisher Scientific, Waltham, MA, USA) as follows: initial denaturation at 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 55°C for 45 seconds, 72°C for 1 minute 15 seconds, and a final extension at 72°C for 8 minutes. Negative controls for extraction and PCR reactions were included, and genomic DNA from Microbial Mock Community B (Staggered, Low Concentration), v5.2L (BEI Resources, Manassas, VA, USA) was included to assess the effect of data processing on observed community content. PCR amplification results were checked by agarose gel electrophoresis and purified from free primers and primer dimer species using AMPure XP beads (Beckman Coulter, USA). Dual indices and Illumina sequencing adapters were attached using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). The final library was cleaned, quantified, normalised and pooled in an equimolar way before sequencing at the Illumina platform at the University of Trento, Trento, Italy. Sequencing was carried out following the manufacturer's recommendations. Sequences were merged, trimmed and filtered using MICCA software (version 1.5.0, Albanese *et al.*, 2015). Overlapping regions of the forward and reverse

sequences that differed by more than eight nucleotides, or did not contain both the forward and reverse PCR primer sequences were discarded. Merged 16S rRNA fragments were discarded if they had an average expected error (AvgEE) greater than 0.1. Operational taxonomic units (OTUs) were assigned using a *de novo* greedy strategy using a cut-off of 97% similarity based on the VSEARCH clustering algorithm (Rognes et al. 2016). Chimeric sequences were discarded. Resulting representatives of each OTU were classified using the Ribosomal Database Project classifier (RDP classifier, version 2.12; Michigan State University [http://rdp.cme.msu.edu/]). Samples that had final read counts of less than 8,000 merged and quality-filtered reads were discarded. The resulting OTUs were analysed at the phylum and class level using Phyloseq version 1.16.2 (McMurdie and Holmes, 2013).

6.3.6 Statistical analyses of helminth development data

A Generalised Linear Mixed Model (GLMM) was used to detect differences in probability of egg hatching of *H. polygyrus* eggs between culture in self and non-self faeces. The response variable was the percentage of successfully hatched eggs in each dish (including replicates). Time (number of culture days), culture type (non-self or self), faecal microbiota alpha diversity of the donor (inverse Simpson index), and of the recipient, plus average helminth burden (EPG of faeces) of the donor, and of the recipient were all fixed variables. To test the effect of sex of donor and recipient on hatch success, a fixed factor was defined as: female donor with female recipient, female donor with male recipient, male donor with female recipient and male donor with male recipient. Culture type with time was a two-way interacting factor. Donor and recipient identity code, as well as culture start date, and culture dish identity were random factors, and the model was weighted by the number of eggs in each culture. A Cox proportional hazards (survival analysis) model was used to test for variation in hatch rate between cultures, where hatch rate was

a response variable and culture type was the independent variable. Egg recovery from *T. muris* cultures was too low (16%, n = 42 eggs from 14/25 cultures) to build a GLMM; instead, a Mann-Whitney U test was used to test for differences in the percentage of embryonated eggs and deteriorated eggs between cultures. GLMM's were built using the 'lme4' package, version 1.1.12 (Bates *et al.*, 2015), while survival analyses were performed in the 'survival' package, version 2.39.5 (Therneau and Grambsch, 2000), in R, version 3.3.2.

6.3.7 Statistical analyses of microbiota data

To determine how OTU abundances varied between egg donors and recipient, OTUs with a differential abundance (i.e., number of reads corrected for sequencing depth) between donors and recipients were first identified, using an approach based on generalised linear models with negative binomial errors implemented in the DESeq2 package (Anders and Huber, 2010). These analyses were run using the default pipeline set-up in DESeq2, and significance values (p >0.05) were derived using likelihood-ratio tests.

In addition, a non-metric multidimensional scaling (NMDS) analysis was used to test for differences in microbiota composition between egg donors and egg recipients. Ecological distances between donors and recipients were assessed using Bray–Curtis dissimilarities (i.e., compositional dissimilarity index that accounts for proportional differences of OTUs among samples) and weighted UniFrac dissimilarity matrices (which accounts both for proportional differences of OTUs and their phylogenetic relatedness; Lozupone and Knight, 2005). OTU tables were scaled before calculation of dissimilarity matrices to achieve an even sequencing depth, corresponding to a minimal number of reads per sample in gut sections or faeces that were included in a given analysis.

6.4 Results

6.4.1 Helminth egg burden of faeces

The faecal yield was sufficient in only 12 out of 14 mice to perform reliable FEC analyses (Appendix A.7, Table A.7.1). *Heligmosomoides polygyrus* was the least prevalent helminth, in 41.7% of mice, and had the lowest mean egg burden compared to the other species found (mean EPG ± standard error = 29.2 ± 7.5). *Trichuris muris* was prevalent in 50.0% of individuals, and had a mean egg burden of 475.5 (± 251.5) EPG. *Hymenolepis* spp. were present in 100% of sampled mice and had the highest mean burden of 1,238.5 (± 273.0) EPG. No eggs from other helminth species were detected in the faeces.

6.4.2 Probability and rate of H. polygyrus *hatching*

The mean hatch success of *H. polygyrus* eggs was significantly higher in non-self (40.3%; ±6.03), compared to self faeces (20.4%; ±6.31; $Z = 2.32$, $p = 0.02$; Figure 6.2). Hatch success of both self and non-self faeces significantly increased with time ($Z = 13.71$, $p < 0.01$), but other factors; alpha diversity of donor microbiota, alpha diversity of recipient microbiota, donor egg burden, recipient egg burden and donor-recipient sex combination, plus the two-way interaction culture type with time, did not significantly affect hatch success ($p > 0.05$). In addition, *H. polygyrus* eggs hatched 1.22 times more quickly in non-self (days 0.5 - 11.5) than in self faeces (days 4.5 - 16.0), although this difference was not significant (Cox proportional hazards model: coef. = 0.20, $p = 0.41$; Figure 6.3).

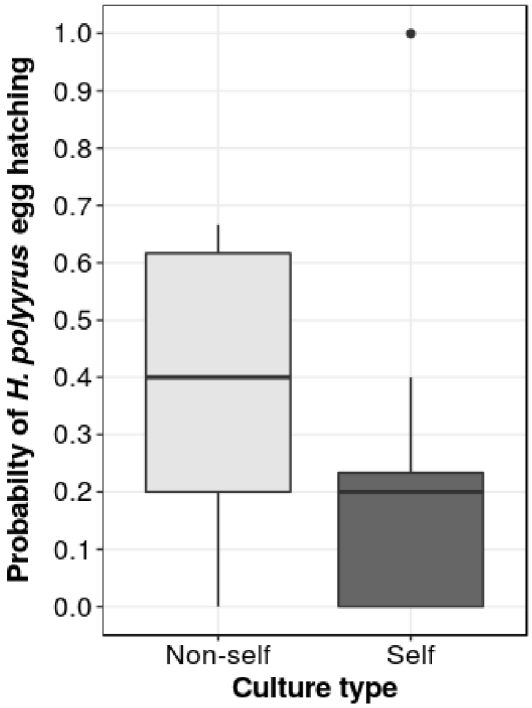

Figure 6.2: Probability of egg hatching of *Heligmosomoides polygyrus* eggs in a transplant experiment, whereby eggs were cultured in 'self' faeces of the host and 'non-self' faeces of a randomly selected individual. Boxes demonstrate the upper and lower quartiles, with median hatching probability indicated. Bars represent the minimum and maximum range of hatching probability.

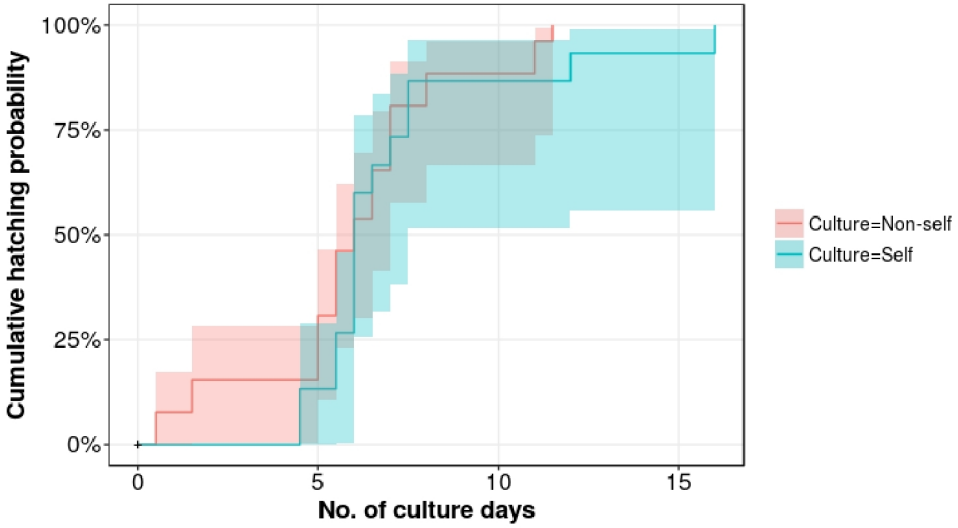

Figure 6.3: Survival plot of cumulative hatching probability of *Heligmosomoides polygyrus* eggs in a transplant experiment, whereby eggs were cultured in 'self' faeces of the host and 'non-self' faeces of a randomly selected individual. Lines represent the expected hatching probability on each day and shaded areas represent the 95% confidence interval.

6.4.3 Probability of T. muris *egg embryonation*

Only 22.1% of *T. muris* eggs in non-self and 12.2% eggs in self faeces were successfully recovered. The mean probability of egg embryonation did not significantly differ between non-self and self faeces (Mann Whitney U: $W = 18$, $p = 0.30$). Of the eggs that were recovered, 94.4% (±3.93) had embryonated in non-self and 100% (±0.00) in self faeces. The mean percentage of deteriorated eggs was significantly higher in non-self compared to self faeces ($W = 100$, $p = 0.05$); 31.5% (± 14.28) exhibited deterioration, of which 58.3% had also embryonated, compared to eggs within self faeces which did not exhibit any deterioration.

6.4.4 Microbiota composition of faeces

It was possible to characterise the faecal microbiota of eight individuals (8 recipients, of which 6 were also donors, Appendix A.7, Table A.7.1). The filtered dataset consisted of 93,909 high-quality reads for eight samples (mean ± standard error = 1,739 ± 1,071, range = 8,074 - 16,153). The mean inverse Simpson index for all samples was 33.0 (± 4.8, range = 17.4 - 59.6). In brief, the faecal microbiota was dominated by *Bacteroidetes* (68.5%), *Firmicutes* (26.3%) and *Proteobacteria* (2.8%), but five other phyla were also identified (Figure 6.4). At the class level, 68.5% of reads belonged to *Bacteroidia* and 20.1% to *Clostridia* (Figure 6.4). Of note, *Tenericutes* (class: *Mollicutes*) was present in the faeces of one individual, which was a recipient but not donor, and *Actinobacteria* (class: *Actinobacteria*) was present in the gut of one individual, which was both a donor and recipient (Figure 6.4).

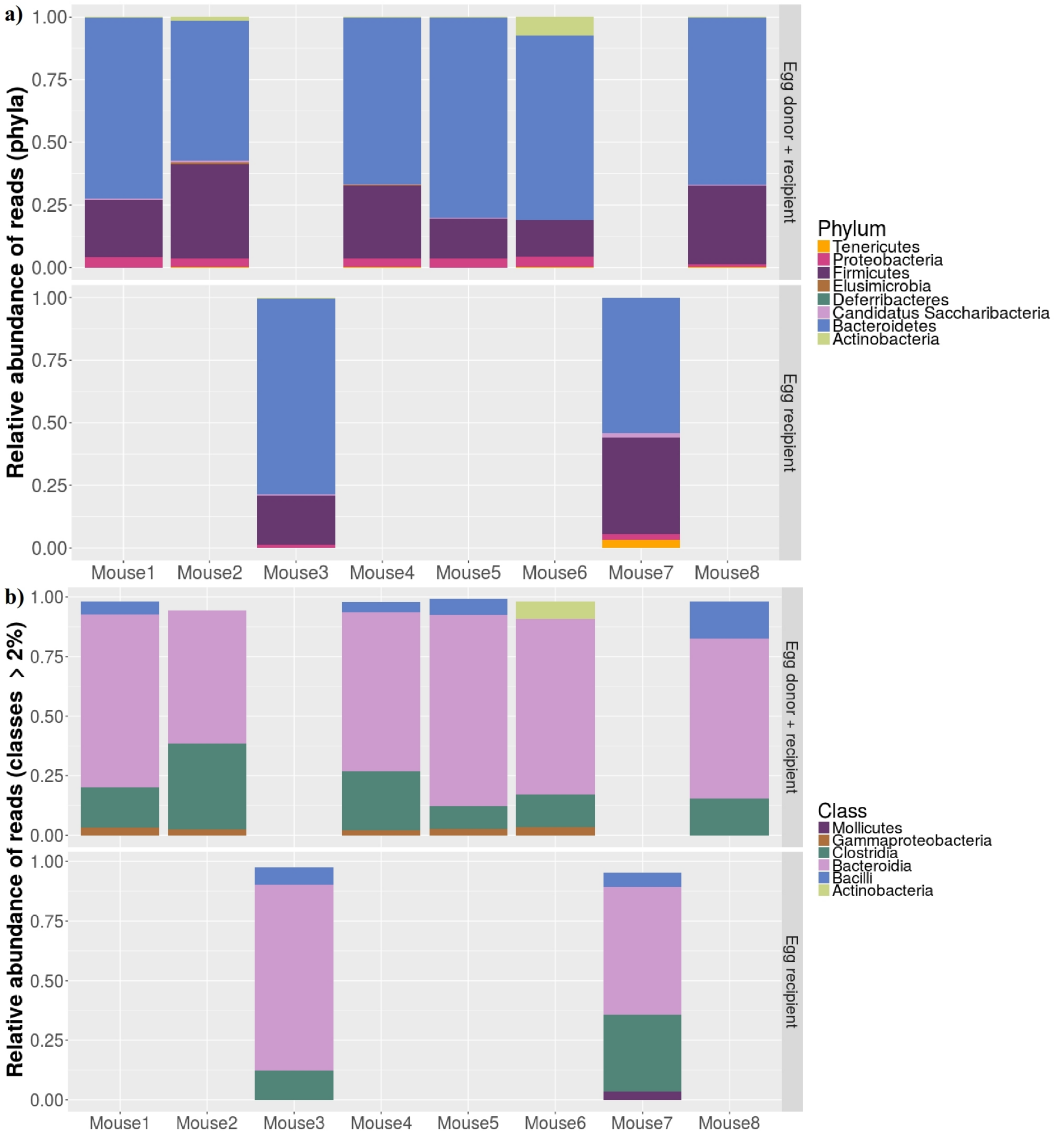

Figure 6.4: Mean proportion of reads of bacterial a) phyla and b) classes (>2%) in faeces of *Apodemus flavicollis* individuals used either as a donor and recipient or only as a recipient of *Heligmosomoides polygyrus* eggs in an egg transplant experiment.

Lachnospiraceae, *Lactobacillaceae* and *Porphyromonadaceae* were all significantly (p <0.05) lower in abundance in the faecal microbiota of individuals used only as recipients, compared to in faeces of individuals used as both donors and recipients (Figure 6.5). However, despite the differences in these specific bacterial families, the overall taxonomic composition of microbiota did not significantly differ between egg donors and recipients, versus individuals which were only

egg recipients, based on both Bray-Curtis (p = 0.38) and weighted UniFrac dissimilarities (p = 0.65; Figure 6.6).

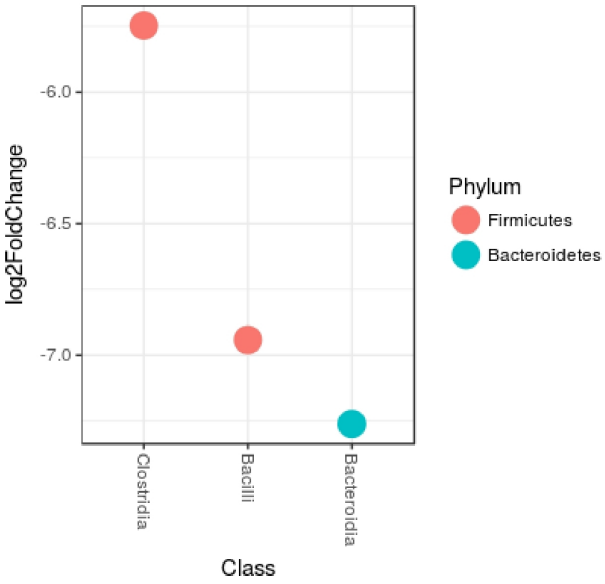

Figure 6.5: OTUs in faecal microbiota that were significantly different in abundance between donor and recipient individuals versus only recipient individuals in an egg transplant experiment with *Heligmosomoides polygyrus* eggs, grouped by microbial class. Briefly, DESeq was used to identify significantly different (p <0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing faecal microbiota between egg donor and recipient individuals, and only egg recipient individuals.

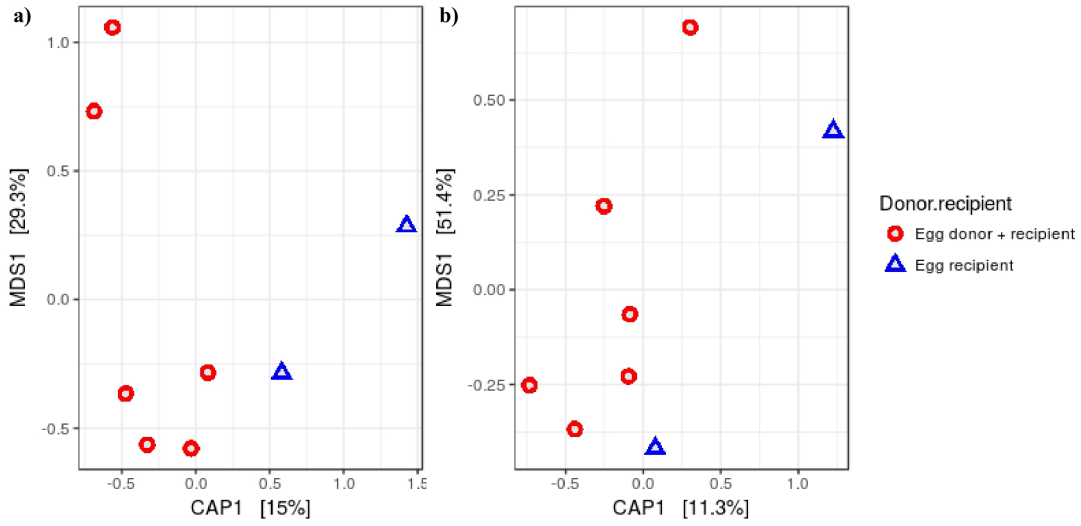

Figure 6.6: Non-metric multidimensional scaling plot of microbiota divergence between faecal samples of individuals used as egg donor and recipients, or only as egg recipients individuals in an egg transplant experiment with *Heligmosomoides polygyrus* eggs based on a) Bray–Curtis (explaining 44.3% variation) and b) weighted UniFrac dissimilarities (explaining 62.7% variation).

6.5 Discussion

In the current study the effect of faecal microbiota on the development of helminth eggs was tested. A transplant of eggs into self and non-self faeces was performed, and differences in the probability of egg hatching and/or rate of development of *H. polygyrus* and *T. muris* eggs between these cultures were tested.

Due to individual and daily variation in egg and faecal yield, not every individual within the experiment was used as both an egg donor and recipient (see Appendix A.7, Table A.7.1 for details). Mean hatch success was significantly higher ($p = 0.02$) in non-self (40.3%) compared to self (20.4%) faeces, suggesting that faecal microbiota from the original egg host has an inhibitory effect on *H. polygyrus* development (Figure 6.2). Unsurprisingly, probability of egg hatching of each culture also significantly increased with time from the start of culture ($p <0.01$). However, other potential influential factors, such as the alpha diversity of faecal microbiota of the egg donor or the recipient microbiota, helminth egg burden of faeces from the donor or the recipient, and sex of the donor and recipient all had no significant affect on hatch success ($p >0.05$), suggesting that natural variation between individuals was not responsible for differences in the probability of egg hatching. As well as having a greater probability of hatching, *H. polygyrus* eggs in non-self faeces hatched at a rate 1.22 times greater than in self faeces; however, this difference was not significant ($p = 0.41$, Figure 6.3). The converse was true for *T. muris*; the probability of egg embryonation was lower in non-self faeces (81.5%) compared to self faeces (100%), but this difference was not significant ($p >0.05$). However, a significantly higher percentage ($p = 0.05$) of *T. muris* eggs recovered from non-self faeces showed visible signs of deterioration (31.5%), while no eggs cultured in self faeces had deteriorated.

Intraspecific gut microbiota composition varies significantly between individuals due to a myriad of host and environmental characteristics (e.g., Lozupone *et al.*, 2012), including due to helminth infection, since both microbiota and helminths share many bi-directional interactions (Glendinning *et al.*, 2014). For example, gut microbiota diversity often increases following helminth infection of the host (Walk *et al.*, 2010; Li *et al.*, 2012; Rausch *et al.*, 2013). It is currently unclear to what extent these subsequent changes in microbial community are a result of indirect microbiota-immunity interplay (Cebra, 1999; Maizels *et al.*, 2004; Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Rausch *et al.*, 2013), or are caused directly by the helminth, for example helminths can secrete antimicrobial products which affect the composition of commensal bacteria (Reynolds *et al.*, 2014). On the other hand, it is clear that some helminth species, including *Trichuris* species and *H. polygyrus*, require contact with specific bacteria to complete their life-cycle (Weinstein *et al.*, 1969; Hayes *et al.*, 2010; Vejzagić *et al.*, 2015a, 2015b). As such, gut microbiota composition of an individual may influence immune phenotypes of helminth development and resistance. In the present study there were significant differences in the abundances of *Lachnospiraceae*, *Lactobacillaceae* and *Porphyromonadaceae* in faecal microbiota of donors and recipients, versus only recipient individuals (which were used only in non-self cultures), wherein these bacterial families were all found in lower abundances in recipient only individuals (Figure 6.5). It is interesting to note that *Lactobacillaceae* decreases host resistance to *H. polygyrus* and *T. muris* (Dea-Ayuela *et al.*, 2008; Reynolds *et al.*, 2014) thus one may expect that individuals with higher abundances of these bacteria may be more susceptible than others to helminth infection. However, these differences in bacterial abundances may have been an artefact of small sample sizes; of the eight individuals which underwent faecal microbiota analysis, just two individuals were only recipients, and the other six were both donors and recipients, thus any differences between the two populations were likely amplified. Despite significant differences in

the abundances of these specific classes of bacteria, the overall taxonomic composition of microbiota was not significantly different between individuals (Bray-Curtis: $p = 0.38$, and weighted UniFrac: $p = 0.65$; Figure 6.6), suggesting that overall microbiota was not responsible for differences in helminth development.

In order to avoid parasitism many hosts have evolved a number of specific and non-specific immune responses that may be mediated by the microbiota to prevent infection (Glendinning *et al.*, 2014; Kabat *et al.*, 2014). Helminth eggs may be affected by these immune responses, which can be stimulated either by the egg itself or by other life stages of the parasite e.g., the adult (Lambert *et al.*, 2015). Immune responses targeted specifically at the egg stage have been recorded in *Schistosoma mansoni* (Pearce *et al.*, 2004), the sheep liver fluke *Fasciola hepatica* (Moxon *et al.*, 2010), and nematodes such as *Strongyloides venezuelensis* (Gonçalves *et al.*, 2012), *Ostertagia circumcincta* (Jørgensen *et al.*, 1998), and other rabbit parasites (Lambert *et al.*, 2015). Antibodies produced by the host, like helminth eggs, can be shed in faeces, and can subsequently bind to the egg and affect development of some helminth species (Jørgensen *et al.*, 1998) but not all (Lambert *et al.*, 2015), which is perhaps why no significant effects on probability of egg development was observed for *T. muris*. Should host antibodies, either present in the faeces or bound to the egg surface, affect *H. polygyrus* egg hatching, external sterilisation of the egg and introduction into new, non-self faeces may release eggs from antibodies and the inhibitory action they induce on egg development, increasing the probability and rate of hatching. However, in self faeces the constraint observed on helminth development could potentially limit self re-infection, a particularly apt adaptation for rodents against parasitism, which engage in coprophagy; a behaviour that can increase the risk of ingesting infective eggs and larvae in faeces (observed in laboratory mice, although there is no evidence for this behaviour in wild *Apodemus flavicollis;*

Ghazal and Avery, 1976).

Although bacteria is involved in the development of multiple helminth species, microbial requirements for development can be specific to the species and even the isolate of the helminth. For example, eggs may be unable to hatch in bacteria from a species which is not the definitive host (Vejzagić *et al.*, 2015a), and each laboratory isolate of *T. muris* responds differently when exposed to certain wild-type bacteria species; some isolates may hatch when cultured with wild-type bacteria (E and E-J isolate), whilst the eggs of the S isolate do not respond to bacteria and can hatch in a sterile environment (Kopper and Mansfield, 2010; Koyama, 2013). The laboratory rodents that host these strains of *T. muris* provide an environment that varies little between host individuals and across generations, due to inbreeding and careful control of external factors such as diet, ambient conditions and host contact with conspecifics in the laboratory. Thus the conditions to which helminth laboratory isolates are subjected to, including host microbiota, remain relatively constant for generations. As such, different isolates of *T. muris* may have evolved specific adaptations to these constant laboratory conditions. As each *T. muris* isolate is passaged through mice with specific immune phenotypes (Johnston *et al.*, 2005) it is possible that variation in hatching requirements is associated with adaptation to the immune phenotype and microbiota of the host. In the present study no significant differences in the embryonation of *T. muris* eggs were observed between self and non-self faeces, suggesting that *T. muris* from wild hosts do not have such specific bacterial requirements for development as their laboratory counterparts (Kopper and Mansfield, 2010; Koyama, 2013). This may be due to the great variation (in terms of genetics, microbiota, immunity, diet, etc.,) between wild host individuals, and even within the same individual between seasons (e.g., Maurice et al. 2015) compared to laboratory rodents. Thus very specific bacterial requirements for hatching are disadvantageous for the

parasite. Indeed, hatching and establishment in laboratory mice of *T. muris* recently isolated from the wild proves difficult, likely because of the disparity in wild and captive mouse microbiota (Hurst and Else, 2013).

In conclusion, the current study suggests that host faeces have an inhibitory effect on hatching of *H. polygyrus* eggs shed within, which may in turn provide the host with some resistance to self re-infection. The ability of faeces to suppress helminth development is not affected by faecal egg burden, nor is it associated with a given faecal microbiota composition or diversity, however it is only effective against eggs shed in faeces by helminths already infecting the host. These results may have implications for helminth control efforts; treatments that alter microbiota composition, e.g., antimicrobials which change faecal microbiota composition (Chapter 4), may alter the ability of faeces inhibit parasite development.

6.6 Author Acknowledgements

The manuscript resulting from this chapter is authored by:

Emily L. Pascoe, Kathryn E. Whittey (Cardiff University), Heidi C. Hauffe (Fondazione Edmund Mach), Julian R. Marchesi (Cardiff University and Imperial College) and Sarah E. Perkins (Cardiff University and Fondazione Edmund Mach).

Chapter 7

General discussion

"He who is not courageous enough to take risks will accomplish nothing in life."

Cassius M. Clay Jr.

Every gut is colonised with a microbiota (Ley *et al.*, 2008), and the vast majority of humans and animals (both wild and domesticated) harbour a parasitic helminth community (macrobiota) composed of at least one species (Hotez *et al.*, 2008; Morgan *et al.*, 2012; Lello *et al.*, 2013). The microbiota and macrobiota have co-infected the gut of both vertebrates and invertebrates throughout evolutionary history, and consequently are likely to interact, both antagonistically and synergistically, with knock-on effects for the host (Glendinning *et al.*, 2014; Reynolds *et al.*, 2015). While some studies have begun to investigate how the microbiota and macrobiota interact (e.g., Hayes *et al.*, 2010; Walk *et al.*, 2010; Cooper *et al.*, 2013; Kreisinger *et al.*, 2015), the number of studies on this topic are currently relatively few, despite a plethora of research on the positive (Round and Mazmanian, 2009; Bilbo *et al.*, 2011) and negative (Tamboli *et al.*, 2004; Sutherland and Scott, 2010; Shetty, 2010) effects on the host of each of these two communities individually. It is therefore pertinent to understand how the microbiota and macrobiota interact, so that future work can extrapolate to the overall effect on host health. Given also, that the gut biome is under increasing evolutionary pressures, for example, excessive, ungoverned and often inappropriate antibiotic and anthelmintic use (Vlassoff *et al.*, 2001; Anadón, 2006; Nielsen, 2009; Vercruysse *et al.*, 2012), and 'Western' diets that deviate from what the human gut has evolved to digest (Hou *et al.*, 2011), it is particularly timely to investigate these interactions so that we can understand the wider implictions on the whole gut biome. Due to recent advances in technologies enabling research on microbial communities (Marchesi and Ravel, 2015), researching microbiota-macrobiota interactions has never been easier. This thesis uses an ecological approach to tease apart some of these microbiota-macrobiota interactions in wild rodent populations, using manipulation as a means to tease apart mechanisms; as advocated by seminal papers in ecology (Paine, 1966).

A review of the gut microbiota literature of animals was performed to provide an overview of the current research landscape (Chapter 2). This review brought to attention the current lack of studies on wild animal gut microbiota. Although studying wild animals can be problematic due to a myriad of logistical and legal restraints (e.g., elusive or rare species which cannot be sampled due to practicalities and laws, and CITES permissions for the translocation of samples collected from endangered species), wild animals can provide insight into natural, intact microbiota composition and functions (Amato, 2013). Gut microbiota studies on wild animals can provide interesting and sometimes surprising insights into the biology of the animal being studied, e.g., myrmecophagous mammals from different evolutionary lineages exhibit striking convergence with respect to gut microbial composition, driven by dietary adaptations (Delsuc *et al.*, 2014), and the giant panda (*Ailuropoda melanoleuca*) relies on gut microbes for cellulose digestion, as its gut is otherwise physiologically adapted instead to a carnivorous diet (Zhu *et al.*, 2011). In addition, wild animal microbiota studies can also provide a model system, which unlike laboratory animals, harbour a diverse microbiota in terms of both the OTUs present and variation observed between individuals, and are exposed to a range of intrinsic and extrinsic factors, rendering the results more 'realistic' and comparable to humans and other species, than laboratory animals. Once again, it could be argued that studying wild animals is difficult, as manipulation is often required in a model system to assign causality and/or directionality of interactions (e.g., Paine, 1966), yet manipulation of wildlife is not always logistically or legally possible. Although sophisticated mathematical and statistical models can be used to assign directionality and causality to interactions (Fenton *et al.*, 2010; Thakar *et al.*, 2012) in a species that cannot be perturbed, this thesis exemplifies the possibilities of manipulating a wild species in order to understand microbiota-macrobiota interactions.

Reviewing the animal gut microbiota literature highlighted that, despite constituting just a fraction of the gut biome (which also includes archaea, viruses, protozoa, fungi and macroparasites), the majority of microbiota research focusses purely on the study of bacteria, with around 12% of studies also investigating at least one other microbial component of the gut biome (Chapter 2). Although studies on bacteria of the gut have shed light on the many functions and interactions of this community (e.g., the gut-brain axis; Aidy *et al.*, 2012), other components of the gut biome, such as the virome and macrobiota, also impact how the microbiota functions and should be given more attention in order to truly understand gut microbiota (Glendinning *et al.*, 2014; Ogilvie and Jones, 2015). Indeed, this thesis has addressed one of these gaps in the literature by studying both the microbiota and macrobiota components of the gut biome, and how they interact.

Given the growing knowledge that helminths and microbiota interact (Glendinning *et al.*, 2014), it is important that we consider the effects of helminth infection on the microbiota. Chapter 3 assessed the effect of anthelmintic treatment on microbiota diversity, composition and OTU abundances. The microbiota of post-treatment individuals remained largely similar to pre-treatment individuals; diversity was not significantly affected, while the taxonomic composition and OTU abundances of only some gut sections, including faeces, were significantly affected. These results suggest that taxonomic composition of microbiota (in the small intestine and caecum), and OTU abundances in some gut sections (small intestine and colon) remain stable following helminth perturbation, a reassuring result given the present-day excessive use of anthelmintics (Vlassoff *et al.*, 2001; Vercruysse *et al.*, 2012). It is possible that a greater effect of anthelmintic treatment on the microbiota was not observed because, although abundance and fecundity of helminths were reduced post-treatment, some helminths did remain in the gut of treated individuals. Another study which claimed to clear completely an experimental helminth

infection (however no data were provided to confirm complete infection clearance) observed a significant shift in microbiota to a composition more similar to uninfected individuals (Houlden *et al.*, 2015). This does raise the controversial question of whether it is necessarily beneficial to eradicate all helminths? While it is true that parasitic infections can have negative impacts on host health, with subsequent detrimental effects on economy, low level infections can be relatively benign (Waller, 2006; Hotez *et al.*, 2008; Shetty, 2010; Sutherland and Scott, 2010; Morgan *et al.*, 2012). Indeed, helminth infections can even have a positive influence on host health and microbiota and can protect against autoimmune diseases (Bilbo *et al.*, 2011). Like any ecosystem, a gut microbiota which shows diversity in taxonomic composition is more likely to be a healthy one (Mosca *et al.*, 2016), and microbiota diversity can increase following helminth infection (Lee *et al.*, 2014), which can even restore a dysbiotic microbiota (Broadhurst *et al.*, 2012). In order to retain the benefits of microbiota composition associated with helminth infection (which could be lost if helminths are completely eradicated, as suggested by Houlden *et al.*, 2015), infected humans and animals could be treated with a conservative dose of anthelmintics, such that some helminths persist and microbiota remains stable. It is important to note, however, that under-dosing is a leading cause of anthelmintic resistance in helminths, as heterozygous resistant individuals may remain and proliferate in the host (Shalaby, 2013). Instead, helminth treatment could avoid current mass drug administration approaches of humans and livestock (Vlassoff *et al.*, 2001; Vercruysse *et al.*, 2012) and target just those individuals showing morbidity associated with infection (although this would require ethical considerations). While Chapter 3 provides initial evidence that microbiota remains largely stable following anthelmintic treatment, the long-term impacts, as well as the effects of higher dosages of anthelmintic more similar to those routinely applied to livestock, should also be considered in future studies.

Although some gut sections were unaffected by anthelmintic, faecal microbiota showed significant shifts in taxonomic composition and OTU abundances following treatment (Chapter 3). Many helminth species (including *H. polygyrus*; Valanparambil *et al.*, 2014, and *T. muris*; Hayes *et al.*, 2010) develop and hatch in host faeces. Host faecal microbiota composition can affect the probability and rate of egg development and hatching (Chapter 6), thus changes in faecal microbiota associated with anthelmintic treatment could have consequences for the numbers of helminth progeny, and thus potentially the perpetuation of helminth infection. To investigate this possibility, helminth eggs could be cultured in faeces from anthelmintic treated individuals, and hatching probability and rate compared with eggs cultured in untreated individuals. Results may establish if reduction in helminth burden associated with anthelmintic treatment is also associated with more successful and quicker development of progeny, which may also increase transmission events. By understanding if the composition of faecal microbiota following anthelmintic infection increases helminth development, strategies could be employed during treatment regimes to avoid further parasite transmission, such as faecal clearing of pastures of recently treated livestock (Corbett *et al.*, 2014).

A potential spin-off from Chapter 3 would be to understand how dietary anthelmintics affect the microbiota. Many species of animal, including primates (Huffman and Seifu, 1989), and ruminants such as sheep (Lisonbee *et al.*, 2009; Villalba *et al.*, 2014), self-medicate during helminth infection, usually by consuming substances rich in tannins. Some dietary compounds, such as tannins, have anthelmintic properties and can decrease nematode abundances and faecal egg counts (Coop and Kyriazakis, 2001; Niezen *et al.*, 2002; Williams *et al.*, 2014). The anthelmintic effect of tannins have been attributed to their protein-binding properties; tannins may bind to proteins in the stomach/rumen, protecting the proteins from degradation so that the host

has more protein available for nutrition, thus potentially strengthening host immune response (Min and Hart, 2003; Min *et al.*, 2004). In addition, tannins may limit the protein available for helminth nutrition or may bind to the helminth larvae cuticle, both of which can lead to helminth death (Athanasiadou *et al.*, 2001). However, as yet it is unknown if the anthelmintic effect of tannins is also linked to changes in microbiota following consumption, which is closely linked to immunity (Round and Mazmanian, 2009). Diet, including tannins (Walenciak *et al.*, 2002) has a rapid and reproducible effect on microbiota (David *et al.*, 2014; Sonnenburg and Bäckhed, 2016), which could in turn effect host resistance to helminths, or effect the microbiota associated with the helminths themselves (see Chapter 5). Using diet as a means to treat helminth infection, e.g., consuming concentrated tannins, could avoid some of the negative impacts associated with treating infection with anthelmintics (e.g., anthelmintic-resistance). Initial investigations have shown that dietary supplementation with chicory roots (which have anthelmintic properties) successfully decreased the burden of one of two helminth species, but the other helminth species present exhibited a higher helminth burden, and no significant changes were reported in microbiota composition (Jensen *et al.*, 2011). However, more research should be conducted on the effect of other tannin-rich foods, or those with anthelmintic properties, on host microbiota to understand the mechanisms and health implications associated with this potential method of helminth treatment.

As well as anthelmintics, antibiotics are also excessively administered to treat infections in humans, livestock and companion animals (Goossens *et al.*, 2005; Prescott, 2008; Landers *et al.*, 2012). A plethora of research has established that antibiotics have significant and often long-lasting impacts on microbiota (Hawrelak and Myers, 2004; Jernberg *et al.*, 2007), and studies from the 1950s suggest that antibiotic treatment may decrease helminth burden and health (Wells,

1951, 1952a, 1952b; Brown, 1952; Chan, 1952; Salem and el-Allaf, 1969; Hoerauf *et al.*, 1999; Saint André *et al.*, 2002). However, these studies investigated the effect of antibiotic on infection with a single helminth species, without taking into consideration the possible subsequent interactions that may occur between coinfecting helminths (Telfer *et al.*, 2010). In Chapter 4, antibiotic treatment was found to have, in general, a negative impact on helminth abundance, but a positive effect on prevalence and fecundity of helminths. Indeed, helminth egg shedding of all species significantly increased. Chapter 4 exemplifies the need for long-term and detailed studies on the effect of antibiotic (and anthelmintic) treatment on components of the gut biome other than those being targeted: although in previous studies antibiotics initially appeared to be an effective method to treat helminth infection (Wells, 1951, 1952a, 1952b; Chan, 1952), implications may include the shedding of more eggs in the environment which could increase possible transmission events. However, although greater in number, eggs shed in the environment may not necessarily be viable; *in utero* egg counts of helminths increased but not significantly so, and it is possible that helminth eggs were spontaneously discharged (Boyce, 1974) following antibiotic treatment, as opposed to antibiotic increasing *in utero* egg production. As such, eggs may have been shed prior to maturation, thus net infectiousness of helminths may not have changed following antibiotic treatment. To confirm infectiousness, eggs shed by helminths from antibiotic treated hosts should be cultured, and the subsequent infective larvae inoculated into hosts, and establishment of infection confirmed.

To progress Chapter 4, the knowledge and methods from Chapter 5 on the basal microbiota associated with helminths could be used to sequence the microbiota of helminths isolated from the guts of antibiotic treated individuals. Comparing the microbiota of helminths from treated and untreated mice could shed light on whether antibiotic treatment of the host effects bacteria in the

helminth, and thus which bacteria may be associated with the increases in helminth egg shedding. In turn, this information could indicate which bacteria within helminths (Chapter 5) are crucial symbionts; those which are removed from the helminths by antibiotic treatment, and therefore linked to reduction in abundance, are likely to have crucial functions within the helminth, which it cannot survive without.

Chapter 5 provided the first characterisation of microbiota associated with multiple species of helminth from naturally infected wild hosts. Alpha diversity of helminths sometimes exceeded that of the gut, and bacterial OTUs were identified in association with helminths that were not found in gut microbiota, implying that helminth microbiota can be acquired from additional sources to the host gut, such as the environment or an intermediate host. It is evident that helminths are associated with a unique microbial composition, which is not randomly acquired (Berg *et al.*, 2016), and future work should pinpoint specific microbial groups that are crucial symbionts of the helminth. This could be achieved by culturing helminths within specific bacterial media and passaging larvae through gnotobiotic mice, and measuring consequent survival and fitness of the helminth. Once identified, crucial bacterial symbionts required for helminth survival could be targeted by antimicrobials to treat helminth infection. However, as results in Chapter 4 demonstrate, antibiotics used to treat helminth infection would have to be carefully selected to avoid subsequent increases in fecundity and potential perpetuation of infection of those helminths remaining following treatment.

Much of the previous work on microbiota-macrobiota interactions have investigated how microbiota of the gut affects helminth infection and development (Weinstein *et al.*, 1969; Bautista-Garfias *et al.*, 2001; Martínez-Gómez *et al.*, 2009; Hayes *et al.*, 2010; Coêlho *et al.*, 2013), with

no consideration paid to the interactions between faecal microbiota and the macrobiota. Chapter 6 aimed to understand if faecal microbiota is an extended immune phenotype of the host and does indeed affect the helminth community, specifically helminth development, by transplanting eggs into faeces from non-self and self individuals and monitoring the probability and rate of egg hatching/development. Results demonstrated that self faecal microbiota of the host provides resistance against helminth egg development, but in faeces from another non-self individual, eggs are freed from these constraints, and the probability and rate of egg hatching is increased. Given that helminths are associated with a microbiota that may be acquired from outside of the host (Chapter 5), and many species of helminth egg are shed and undergo development within faeces (Hayes *et al.*, 2010; Valanparambil *et al.*, 2014), it is not surprising that faecal microbiota does affect helminth egg development and hatching. These findings may have implications for helminth control efforts; treatments that alter faecal microbiota composition, e.g., anthelmintics (Chapter 3) or antibiotics (Chapter 4), may alter the ability of faeces to inhibit parasite development. As increased hatching probability and rate may equate to more progeny, this could increase the chance of transmission events, with negative impacts on the host population. As such, studying the effect of helminth development in faeces from an anthelmintic or antibiotic treated host may indicate if certain precautions should be made following these treatments to avoid increased helminth transmission, e.g., faeces removal from pastures (Corbett *et al.*, 2014).

To conclude, this thesis identifies the need for animal gut microbiota research to progress to the study of wild animals, with natural and intact microbiota (Chapter 2). It was demonstrated within this thesis that perturbation of either the microbiota or macrobiota has wider implications on other components of the gut biome; anthelmintic treatment was associated with significant changes in taxonomic composition of faeces microbiota and the OTU abundances therein (Chapter 3).

Moreover, antibiotic treatment was associated with significant increases in helminth egg shedding (Chapter 4). In addition, the microbiota associated with helminths was characterised, providing the first steps to identifying possible symbionts that could be targeted for removal to treat helminth infections (Chapter 5). Finally, the effect of faecal microbiota on helminth development was investigated, with results indicating that faecal microbiota from infected hosts can be self-limiting to helminth development (Chapter 6). Future work should combine the knowledge from Chapter 5 on helminth microbiota with the effects seen following microbiota and macrobiota perturbation to tease apart how these perturbations may function and identify helminth symbionts. In addition, long-term studies of microbiota-macrobiota interactions would be beneficial to determine the net effect of such perturbations, for example whilst in the short-term antibiotic decreases helminth abundances, treatment also increases egg output of helminths which in the longer term could lead to more progeny and increased chances of transmission events.

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Appendix A.1: Supplementary table of data presented in Chapter 2

Table A.1.1: Details of the 650 recently published (2009-2016) non-human animal gut microbiota studies randomly selected for review, and the corresponding data that were extracted from each article.

Animal group	Data collection method	Taxonomic group	Research question 1	Research question 2	Research question 3	Research question 4	Research question 5	Target microbes	Reference
Model	Perturbation	Bird	Prebiotic	N/A	N/A	N/A	N/A	Bacteria	(Abd El-Khalek *et al.*, 2012)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Abdel-Wareth *et al.*, 2012)
Model	Perturbation	Mammal	Immunity	Non-infectious disease	Antibiotic	Bacterial transplant	N/A	Bacteria	(Abdollahi-Roodsaz *et al.*, 2014)
Wild	Observation	Insect	Vertical transmission	Diet	N/A	N/A	N/A	Bacteria & archaea	(Abdul Rahman *et al.*, 2015)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria & archaea	(Abecia *et al.*, 2013)
Domestic	Perturbation	Fish	Age	Genotype	N/A	N/A	N/A	Bacteria	(Aguilera *et al.*, 2013)
Domestic	Perturbation	Mammal	Probiotic	N/A	N/A	N/A	N/A	Bacteria, fungi & protozoa	(Ahmed *et al.*, 2014)
Model	Perturbation	Mammal	Bacterial transplant	Gut-brain axis	Development	N/A	N/A	Bacteria	(Aidy *et al.*, 2012)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Akbarian *et al.*, 2014)
Wild	Observation	Insect	Community composition	Interspecific comparison	N/A	N/A	N/A	Bacteria	(Aksoy *et al.*, 2014)
Domestic	Perturbation	Fish	Prebiotic	Production	N/A	N/A	N/A	Bacteria	(Akter *et al.*, 2016)
Model	Perturbation	Mammal	Development	Bacterial transplant	N/A	N/A	N/A	Bacteria	(Al-Asmakh *et al.*, 2014)
Model	Perturbation	Mammal	Non-	Antibiotic	N/A	N/A	N/A	Bacteria	(Alkanani *et al.*, 2014)

			infectious disease						
Wild	Perturbation	Mammal	Environment	Diet	N/A	N/A	N/A	Bacteria	(Amato *et al.*, 2013)
Domestic	Perturbation	Bird	Diet	Production	Development	N/A	N/A	Bacteria	(Amerah *et al.*, 2011)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria, funghi & protozoa	(Anantasook *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria	(Andersen *et al.*, 2011)
Model	Perturbation	Mammal	Probiotic	Growth	Immunity	N/A	N/A	Bacteria	(Angelakis *et al.*, 2012)
Domestic	Perturbation	Non-insect invertebrate	Production	Diet	Immunity	N/A	N/A	Bacteria	(Anuta *et al.*, 2011)
Wild	Perturbation	Mammal	Prebiotic	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Ardeshir *et al.*, 2014)
Wild	Observation	Insect	Age	Diet	N/A	N/A	N/A	Bacteria	(Arias-Cordero *et al.*, 2012)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Arimatsu *et al.*, 2014)
Model	Perturbation	Mammal	Infectious disease	Diet	N/A	N/A	N/A	Bacteria	(Arrazuria *et al.*, 2016)
Domestic	Perturbation	Fish	Probiotic	Interspecific comparison	Production	N/A	N/A	Bacteria	(Askarian *et al.*, 2011)
Model	Perturbation	Mammal	Non-infectious disease	Prebiotic	Probiotic	Immunity	Synbiotic	Bacteria	(Axling *et al.*, 2012)
Domestic	Observation	Fish	Domestication	N/A	N/A	N/A	N/A	Bacteria	(Bacanu and Oprea, 2013)
Model	Observation	Mammal	Gut-brain axis	Immunity	N/A	N/A	N/A	Bacteria	(Bailey *et al.*, 2010)
Model	Perturbation	Mammal	Gut-brain	Immunity	Antibiotic	N/A	N/A	Bacteria	(Bailey *et al.*, 2011)

			axis						
Domestic	Perturbation	Mammal	Probiotic	Production	Vertical transmission	N/A	N/A	Bacteria	(Baker *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Baldwin *et al.*, 2016)
Model	Perturbation	Mammal	Domestication	Behaviour	Gut-brain axis	Immunity	N/A	Bacteria	(Bangsgaard Bendtsen *et al.*, 2012)
Model	Perturbation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria & archaea	(Barfod *et al.*, 2013)
Model	Perturbation	Mammal	Probiotic	Domestication	Immunity	Gut-brain axis	N/A	Bacteria	(Barouei *et al.*, 2012)
Model	Perturbation	Mammal	Vertical transmission	Genotype	Diet	N/A	N/A	Bacteria	(Barron Pastor and Gordon, 2016)
Domestic	Perturbation	Fish	Immunity	Diet	Genotype	Production	N/A	Bacteria	(Batista *et al.*, 2016)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Baurhoo *et al.*, 2011)
Model	Perturbation	Mammal	Non-infectious disease	Antibiotic	N/A	N/A	N/A	Bacteria	(Bazett *et al.*, 2016)
Domestic	Perturbation	Mammal	Infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Bearson *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	Bacterial transplant	N/A	N/A	N/A	Bacteria, funghi & protozoa	(Belanche *et al.*, 2012)
Model	Perturbation	Mammal	Diet	Non-infectious disease	Immunity	N/A	N/A	Bacteria	(Belcheva *et al.*, 2014)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria	(Bennett *et al.*, 2013)
Model	Perturbation	Mammal	Infectious disease	Immunity	Genotype	N/A	N/A	Bacteria	(Bereswill *et al.*, 2014)
Domestic	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria & viruses	(Berg Miller *et al.*, 2012)

Model	Perturbation	Mammal	Diet	Antibiotic	Non-infectious disease	N/A	N/A	Bacteria	(Bhat and Al-daihan, 2016)
Domestic	Observation	Fish	Diet	Interspecific comparison	N/A	N/A	N/A	Bacteria	(Bolnick *et al.*, 2014)
Model	Perturbation	Mammal	Prebiotic	Probiotic	Non-infectious disease	Immunity	Synbiotic	Bacteria	(Bomhof *et al.*, 2014)
Model	Perturbation	Mammal	Non-infectious disease	Antibiotic	Immunity	N/A	N/A	Bacteria	(Bongers *et al.*, 2014)
Domestic	Perturbation	Bird	Prebiotic	Community composition	N/A	N/A	N/A	Bacteria	(Bonos *et al.*, 2011)
Domestic	Perturbation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Borewicz *et al.*, 2015)
Domestic	Perturbation	Bird	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Boroojeni *et al.*, 2014)
Domestic	Perturbation	Bird	Diet	Infectious disease	Production	Antibiotic	N/A	Bacteria	(Bortoluzzi *et al.*, 2015)
Domestic	Perturbation	Mammal	Antibiotic	Production	Immunity	N/A	N/A	Bacteria	(Bosi *et al.*, 2011)
Model	Perturbation	Mammal	Toxicology	N/A	N/A	N/A	N/A	Bacteria	(Breton *et al.*, 2013)
Model	Perturbation	Mammal	Genotype	Immunity	N/A	N/A	N/A	Bacteria	(Brinkman *et al.*, 2011)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	Genotype	N/A	N/A	Bacteria	(Brinkman *et al.*, 2013)
Model	Perturbation	Mammal	Infectious disease	Non-infectious disease	Immunity	N/A	N/A	Bacteria	(Broadhurst *et al.*, 2012)
Model	Observation	Insect	Genotype	Age	Immunity	Diet	N/A	Bacteria	(Broderick *et al.*, 2014)
Model	Perturbation	Mammal	Probiotic	Diet	Non-	Immunity	N/A	Bacteria	(Bull-Otterson *et al.*, 2013)

					infectious disease				
Domestic	Perturbation	Mammal	Toxicology	Infectious disease	Immunity	N/A	N/A	Bacteria	(Burel *et al.*, 2013)
Domestic	Perturbation	Fish	Production	Diet	Prebiotic	N/A	N/A	Bacteria	(Burr *et al.*, 2010)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Buzoianu *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	Vertical transmission	N/A	N/A	N/A	Bacteria	(Buzoianu *et al.*, 2013)
Model	Perturbation	Mammal	Genotype	Environment	N/A	N/A	N/A	Bacteria	(Campbell *et al.*, 2012)
Model	Perturbation	Mammal	Exercise	Diet	Immunity	N/A	N/A	Bacteria	(Campbell *et al.*, 2016)
Model	Perturbation	Mammal	Non-infectious disease	Toxicology	Diet	Immunity	N/A	Bacteria	(Canesso *et al.*, 2014)
Domestic	Observation	Fish	Genotype	N/A	N/A	N/A	N/A	Bacteria	(Cantas *et al.*, 2011)
Model	Observation	Fish	Age	Environment	Community composition	N/A	N/A	Bacteria	(Cantas *et al.*, 2012)
Model	Perturbation	Fish	Diet	N/A	N/A	N/A	N/A	Bacteria	(Cao *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Cao *et al.*, 2016a)
Model	Perturbation	Mammal	Non-infectious disease	Diet	Immunity	N/A	N/A	Bacteria	(Cao *et al.*, 2016b)
Wild	Observation	Mammal	Community composition	Temporal	Diet	N/A	N/A	Bacteria	(Carey *et al.*, 2013)
Model	Perturbation	Mammal	Immunity	Antibiotic	N/A	N/A	N/A	Bacteria	(Carvalho *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Castillo-Lopez *et al.*, 2014)
Wild	Perturbation	Insect	Infectious disease	Antibiotic	N/A	N/A	N/A	Bacteria	(Castro *et al.*, 2012a)

Wild	Perturbation	Insect	Drugs	Infectious disease	N/A	N/A	N/A	Bacteria	(Castro *et al.*, 2012b)
Domestic	Perturbation	Fish	Probiotic	Diet	Immunity	N/A	N/A	Bacteria	(Cerezuela *et al.*, 2012)
Domestic	Perturbation	Fish	Diet	Probiotic	N/A	N/A	N/A	Bacteria	(Cerezuela *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Chaplin *et al.*, 2016)
Domestic	Perturbation	Fish	Diet	Immunity	Production	N/A	N/A	Bacteria	(Chen *et al.*, 2014a)
Domestic	Perturbation	Fish	Diet	Immunity	Production	N/A	N/A	Bacteria	(Chen *et al.*, 2014b)
Model	Perturbation	Mammal	Probiotic	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Chen *et al.*, 2014c)
Model	Observation	Mammal	Methods	Community composition	N/A	N/A	N/A	Bacteria, archaea, funghi & protozoa	(Chen *et al.*, 2015)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Chen *et al.*, 2016)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria & funghi	(Cherdthong and Wanapat, 2013)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria, funghi & protozoa	(Cherdthong *et al.*, 2015)
Wild	Observation	Mammal	Community composition	Vertical transmission	N/A	N/A	N/A	Bacteria	(Chhour *et al.*, 2010)
Domestic	Perturbation	Mammal	Probiotic	Production	Metabolism	N/A	N/A	Bacteria & protozoa	(Chiquette *et al.*, 2015)
Model	Perturbation	Mammal	Synbiotic	Non-infectious disease	Immunity	N/A	N/A	Bacteria	(Chiu *et al.*, 2015)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Cho *et al.*, 2014)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Choe *et al.*, 2012)

Model	Observation	Insect	Age	Function	Immunity	Genotype	N/A	Bacteria	(Clark *et al.*, 2015)
Wild	Observation	Mammal	Infectious disease	Community composition	N/A	N/A	N/A	Bacteria	(Coldham *et al.*, 2013)
Model	Perturbation	Mammal	Non-infectious disease	Diet	Growth	N/A	N/A	Bacteria	(Collins *et al.*, 2015)
Model	Observation	Mammal	Age	N/A	N/A	N/A	N/A	Bacteria & archaea	(Combes *et al.*, 2011)
Wild	Perturbation	Insect	Interspecific comparison	Development	N/A	N/A	N/A	Bacteria	(Coon *et al.*, 2014)
Domestic	Perturbation	Fish	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Cordero *et al.*, 2015)
Domestic	Perturbation	Mammal	Infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Costa *et al.*, 2014)
Domestic	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Costa *et al.*, 2015a)
Domestic	Perturbation	Mammal	Antibiotic	N/A	N/A	N/A	N/A	Bacteria	(Costa *et al.*, 2015b)
Model	Observation	Reptile	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Costello *et al.*, 2010)
Model	Perturbation	Mammal	Diet	Metabolism	N/A	N/A	N/A	Bacteria	(Cox *et al.*, 2013)
Domestic	Perturbation	Bird	Environment	N/A	N/A	N/A	N/A	Bacteria	(Cressman *et al.*, 2010)
Model	Perturbation	Mammal	Behaviour	Gut-brain axis	N/A	N/A	N/A	Bacteria	(Crumeyrolle-Arias *et al.*, 2014)
Domestic	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria & archaea	(Cunha *et al.*, 2011)
Domestic	Perturbation	Bird	Antibiotic	Diet	Production	N/A	N/A	Bacteria	(Czerwiński *et al.*, 2012)
Model	Perturbation	Mammal	Non-infectious disease	Probiotic	Prebiotic	N/A	N/A	Bacteria	(D'Argenio *et al.*, 2013)
Domestic	Observation	Mammal	Community	N/A	N/A	N/A	N/A	Bacteria	(Dai *et al.*, 2012)

			composition						
Model	Perturbation	Mammal	Diet	Metabolism	N/A	N/A	N/A	Bacteria	(Daniel *et al.*, 2014)
Domestic	Perturbation	Non-insect invertebrate	Prebiotic	Production	Probiotic	Synbiotic	N/A	Bacteria	(Daniels *et al.*, 2010)
Domestic	Observation	Bird	Genotype	Production	Temporal	N/A	N/A	Bacteria	(Danzeisen *et al.*, 2013)
Domestic	Perturbation	Mammal	Domesticatio n	Immunity	N/A	N/A	N/A	Bacteria	(Davis *et al.*, 2010)
Domestic	Perturbation	Fish	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Dawood *et al.*, 2016)
Domestic	Observation	Mammal	Genotype	Production	N/A	N/A	N/A	Bacteria	(De Barbieri *et al.*, 2015)
Model	Perturbation	Mammal	Diet	Genotype	Non-infectious disease	Immunity	N/A	Bacteria	(de La Serre *et al.*, 2010)
Domestic	Perturbation	Mammal	Diet	Prebiotic	N/A	N/A	N/A	Bacteria	(De Nardi *et al.*, 2016)
Domestic	Perturbation	Fish	Diet	N/A	N/A	N/A	N/A	Bacteria	(de Paula Silva *et al.*, 2011)
Model	Perturbation	Mammal	Non-infectious disease	Diet	N/A	N/A	N/A	Bacteria	(de Wit *et al.*, 2012)
Wild	Observation	Mammal	Genotype	Environment	N/A	N/A	N/A	Bacteria	(Degnan *et al.*, 2012)
Wild	Observation	Mammal	Phylogeny	Diet	N/A	N/A	N/A	Bacteria	(Delsuc *et al.*, 2014)
Wild	Perturbation	Insect	Age	Environment	N/A	N/A	N/A	Bacteria & funghi	(Dematheis *et al.*, 2012)
Domestic	Perturbation	Mammal	Infectious disease	Community composition	Metabolism	N/A	N/A	Bacteria	(Derakhshani *et al.*, 2016)
Domestic	Perturbation	Fish	Diet	N/A	N/A	N/A	N/A	Bacteria	(Desai *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria, archaea & viruses	(Deusch *et al.*, 2014)
Model	Perturbation	Mammal	Antibiotic	Surgical procedure	N/A	N/A	N/A	Bacteria	(Devine *et al.*, 2013)

Wild	Observation	Bird	Interspecific comparison	N/A	N/A	N/A	N/A	Bacteria	(Dewar *et al.*, 2014a)
Wild	Observation	Bird	Diet	Interspecific comparison	Temporal	N/A	N/A	Bacteria	(Dewar *et al.*, 2014b)
Domestic	Perturbation	Fish	Domesticatio n	Diet	N/A	N/A	N/A	Bacteria	(Dhanasiri *et al.*, 2011)
Domestic	Perturbation	Mammal	Diet	Age	N/A	N/A	N/A	Bacteria	(Dicksved *et al.*, 2015)
Wild	Observation	Insect	Interspecific comparison	Phylogeny	N/A	N/A	N/A	Bacteria	(Dietrich *et al.*, 2014)
Wild	Observation	Mammal	Diet	Interspecific comparison	Community composition	N/A	N/A	Bacteria	(Dill-McFarland *et al.*, 2016)
Model	Perturbation	Mammal	Genotype	Immunity	N/A	N/A	N/A	Bacteria	(Dimitriu *et al.*, 2013)
Domestic	Observation	Fish	Prebiotic	Diet	Production	N/A	N/A	Bacteria	(Dimitroglou *et al.*, 2010)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria, funghi & protozoa	(Ding *et al.*, 2014)
Model	Observation	Non-insect invertebrate	Community composition	Environment	N/A	N/A	N/A	Bacteria	(Dishaw *et al.*, 2014)
Model	Perturbation	Mammal	Probiotic	Age	Gut-brain axis	N/A	N/A	Bacteria	(Distrutti *et al.*, 2014)
Wild	Perturbation	Non-insect invertebrate	Diet	N/A	N/A	N/A	N/A	Bacteria	(Dittmer *et al.*, 2012)
Model	Perturbation	Mammal	Probiotic	Diet	Non-infectious disease	Immunity	N/A	Bacteria	(Dolpady *et al.*, 2016)
Domestic	Perturbation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Drumo *et al.*, 2015)
Wild	Observation	Invertebrate	Community composition	N/A	N/A	N/A	N/A	Bacteria & archaea	(Dudek *et al.*, 2014)

Wild	Observation	Non-insect invertebrate	Environment	Community composition	N/A	N/A	N/A	Bacteria & archaea	(Durand *et al.*, 2010)
Domestic	Perturbation	Bird	Probiotic	Production	Immunity	Environment	N/A	Bacteria & funghi	(Elangovan *et al.*, 2011)
Model	Perturbation	Mammal	Non-infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Ellekilde *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria & archaea	(Ellison *et al.*, 2014)
Domestic	Perturbation	Bird	Diet	Production	Infectious disease	Immunity	N/A	Bacteria	(Engberg *et al.*, 2012)
Wild	Perturbation	Insect	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Engel *et al.*, 2012)
Model	Perturbation	Mammal	Prebiotic	N/A	N/A	N/A	N/A	Bacteria	(Engevik *et al.*, 2013)
Model	Perturbation	Mammal	Drugs	N/A	N/A	N/A	N/A	Bacteria	(Eshar and Weese, 2014)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Espley *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Antibiotic	Non-infectious disease	N/A	N/A	Bacteria	(Esposito *et al.*, 2015)
Model	Perturbation	Mammal	Diet	Exercise	N/A	N/A	N/A	Bacteria	(Evans *et al.*, 2014)
Model	Perturbation	Mammal	Prebiotic	Non-infectious disease	Genotype	N/A	N/A	Bacteria	(Everard *et al.*, 2011)
Domestic	Perturbation	Fish	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Feng *et al.*, 2011)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Feng *et al.*, 2015)
Domestic	Perturbation	Fish	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Ferguson *et al.*, 2010)
Domestic	Perturbation	Mammal	Diet	Methods	N/A	N/A	N/A	Bacteria & archaea	(Fernando *et al.*, 2010)

Model	Perturbation	Mammal	Antibiotic	Infectious disease	Immunity	N/A	N/A	Bacteria	(Ferreira *et al.*, 2011)
Domestic	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Fiesel *et al.*, 2014)
Domestic	Observation	Fish	Community composition	Genotype	Environment	N/A	N/A	Bacteria	(Fjellheim *et al.*, 2012)
Model	Perturbation	Mammal	Non-infectious disease	Diet	N/A	N/A	N/A	Bacteria	(Fleissner *et al.*, 2010)
Wild	Observation	Mammal	Environment	Interspecific comparison	Diet	N/A	N/A	Bacteria & archaea	(Fogel, 2015)
Domestic	Perturbation	Bird	Probiotic	Production	Age	N/A	N/A	Bacteria	(Fonseca *et al.*, 2010)
Model	Observation	Fish	Temporal	N/A	N/A	N/A	N/A	Bacteria	(Fortes-Silva *et al.*, 2016)
Wild	Observation	Fish	Interspecific comparison	Environment	N/A	N/A	N/A	Bacteria	(Franchini *et al.*, 2014)
Domestic	Observation	Mammal	Genotype	Community composition	N/A	N/A	N/A	Bacteria, archaea & protozoa	(Frey *et al.*, 2010)
Domestic	Perturbation	Mammal	Synbiotic	Production	Probiotic	Prebiotic	N/A	Bacteria	(Frizzo *et al.*, 2011)
Domestic	Perturbation	Mammal	Infectious disease	Immunity	Genotype	N/A	N/A	Bacteria	(Gao *et al.*, 2013)
Domestic	Observation	Mammal	Drugs	Community composition	N/A	N/A	N/A	Bacteria	(Garcia-Mazcorro *et al.*, 2012)
Model	Perturbation	Mammal	Genotype	Non-infectious disease	Vertical transmission	N/A	N/A	Bacteria	(Garrett *et al.*, 2010)
Domestic	Perturbation	Fish	Diet	N/A	N/A	N/A	N/A	Bacteria	(Gatesoupe *et al.*, 2014)
Domestic	Perturbation	Fish	Probiotic	Production	N/A	N/A	N/A	Bacteria	(Geraylou *et al.*, 2013a)
Domestic	Perturbation	Fish	Prebiotic	Production	N/A	N/A	N/A	Bacteria	(Geraylou *et al.*, 2013b)
Domestic	Perturbation	Fish	Diet	Metabolism	Production	N/A	N/A	Bacteria &	(Geurden *et al.*, 2014)

								funghi	
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Ghaffarzadegan *et al.*, 2016)
Domestic	Perturbation	Bird	Production	Diet	N/A	N/A	N/A	Bacteria	(Ghazaghi *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Age	Non-infectious disease	Immunity	N/A	Bacteria	(Ghosh *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria & protozoa	(Giannenas *et al.*, 2011a)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Giannenas *et al.*, 2011b)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Giannenas *et al.*, 2014)
Domestic	Perturbation	Fish	Environment	N/A	N/A	N/A	N/A	Bacteria	(Giatsis *et al.*, 2014)
Domestic	Perturbation	Fish	Environment	Diet	N/A	N/A	N/A	Bacteria	(Giatsis *et al.*, 2015)
Model	Perturbation	Mammal	Infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Gill *et al.*, 2012)
Domestic	Perturbation	Fish	Probiotic	Production	Immunity	N/A	N/A	Bacteria	(Gisbert *et al.*, 2013)
Wild	Perturbation	Non-insect invertebrate	Community composition	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Givens *et al.*, 2013)
Domestic	Observation	Fish	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Godoy *et al.*, 2015)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	Toxicology	N/A	N/A	Bacteria	(Gómez-Hurtado *et al.*, 2011)
Domestic	Perturbation	Fish	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Green *et al.*, 2013)
Wild	Observation	Insect	Community composition	N/A	N/A	N/A	N/A	Bacteria & archaea	(Grieco *et al.*, 2013)
Domestic	Perturbation	Fish	Prebiotic	Environment	Function	N/A	N/A	Bacteria	(Guerreiro *et al.*, 2016)
Model	Perturbation	Mammal	Genotype	Immunity	N/A	N/A	N/A	Bacteria	(Gulati *et al.*, 2012)

Wild	Observation	Insect	Infectious disease	Community composition	N/A	N/A	N/A	Bacteria	(Gumiel *et al.*, 2015)
Model	Perturbation	Mammal	Diet	Toxicology	N/A	N/A	N/A	Bacteria	(Guo *et al.*, 2014a)
Model	Perturbation	Mammal	Toxicology	N/A	N/A	N/A	N/A	Bacteria	(Guo *et al.*, 2014b)
Domestic	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Haenen *et al.*, 2013)
Domestic	Observation	Mammal	Infectious disease	Temporal	N/A	N/A	N/A	Bacteria & archaea	(Haley *et al.*, 2016)
Model	Perturbation	Mammal	Synbiotic	Antibiotic	N/A	N/A	N/A	Bacteria	(Hammami *et al.*, 2015)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Han *et al.*, 2010)
Domestic	Perturbation	Bird	Probiotic	Antibiotic	Production	N/A	N/A	Bacteria	(Han *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Age	N/A	N/A	N/A	Bacteria	(Han *et al.*, 2014)
Model	Perturbation	Mammal	Antibiotic	Immunity	Prebiotic	N/A	N/A	Bacteria	(Hansen *et al.*, 2013)
Domestic	Perturbation	Fish	Diet	Production	N/A	N/A	N/A	Bacteria	(Hartviksen *et al.*, 2014)
Domestic	Perturbation	Fish	Antibiotic	Genotype	N/A	N/A	N/A	Bacteria	(He *et al.*, 2010)
Model	Perturbation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(He *et al.*, 2012a)
Domestic	Perturbation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(He *et al.*, 2012b)
Model	Perturbation	Mammal	Bacterial transplant	Genotype	Infectious disease	N/A	N/A	Bacteria	(Heimesaat *et al.*, 2013)
Model	Perturbation	Mammal	Infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Heimesaat *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Non-infectious disease	Immunity	N/A	N/A	Bacteria	(Heyman-Lindén *et al.*, 2016)
Wild	Observation	Bird	Genotype	Environment	Age	Diet	N/A	Bacteria	(Hird *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Non-	N/A	N/A	N/A	Bacteria	(Holm *et al.*, 2016)

				infectious disease					
Domestic	Perturbation	Bird	Antibiotic	Diet	Immunity	Production	N/A	Bacteria	(Hong *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	Metabolism	N/A	N/A	N/A	Bacteria	(Hooda *et al.*, 2013)
Domestic	Perturbation	Fish	Diet	Production	Immunity	N/A	N/A	Bacteria	(Hoseinifar *et al.*, 2011)
Domestic	Perturbation	Fish	Prebiotic	Production	N/A	N/A	N/A	Bacteria	(Hoseinifar *et al.*, 2013)
Domestic	Perturbation	Fish	Prebiotic	Immunity	Production	N/A	N/A	Bacteria	(Hoseinifar *et al.*, 2014a)
Domestic	Perturbation	Fish	Diet	Production	Immunity	Prebiotic	N/A	Bacteria	(Hoseinifar *et al.*, 2014b)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria	(Hosseintabar *et al.*, 2014)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Hu *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Huang *et al.*, 2013)
Model	Observation	Mammal	Genotype	N/A	N/A	N/A	N/A	Bacteria	(Hufeldt *et al.*, 2010a)
Model	Observation	Mammal	Genotype	Environment	N/A	N/A	N/A	Bacteria	(Hufeldt *et al.*, 2010b)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria	(Huws *et al.*, 2012)
Model	Perturbation	Mammal	Antibiotic	Non-infectious disease	Metabolism	N/A	N/A	Bacteria	(Hwang *et al.*, 2015)
Domestic	Perturbation	Non-insect invertebrate	Probiotic	N/A	N/A	N/A	N/A	Bacteria	(Iehata *et al.*, 2014)
Domestic	Perturbation	Mammal	Antibiotic	N/A	N/A	N/A	N/A	Bacteria	(Igarashi *et al.*, 2014)
Wild	Observation	Mammal	Diet	Community composition	Age	N/A	N/A	Bacteria & archaea	(Ilmberger *et al.*, 2014)
Model	Perturbation	Mammal	Drugs	N/A	N/A	N/A	N/A	Bacteria	(Imaeda *et al.*, 2012)
Model	Observation	Mammal	Methods	N/A	N/A	N/A	N/A	Bacteria	(Indugu *et al.*, 2016)
Domestic	Perturbation	Fish	Diet	Probiotic	N/A	N/A	N/A	Bacteria	(Ingerslev *et al.*, 2014)
Domestic	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Ishaq and Wright, 2012)

Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Islam *et al.*, 2011)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria	(Jahanpour *et al.*, 2014)
Model	Perturbation	Insect	Infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Jakubowska *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria	(Jami *et al.*, 2014)
Domestic	Observation	Mammal	Diet	Environment	N/A	N/A	N/A	Bacteria	(Janczyk *et al.*, 2010)
Domestic	Perturbation	Mammal	Diet	Domestication	N/A	N/A	N/A	Bacteria	(Jansman *et al.*, 2012)
Model	Perturbation	Mammal	Antibiotic	Diet	Non-infectious disease	N/A	N/A	Bacteria	(Jena *et al.*, 2014)
Domestic	Perturbation	Mammal	Prebiotic	Infectious disease	N/A	N/A	N/A	Bacteria	(Jensen *et al.*, 2011)
Model	Perturbation	Mammal	Diet	Growth	Immunity	N/A	N/A	Bacteria	(Jiang *et al.*, 2016)
Model	Observation	Insect	Age	Immunity	Bacterial interference	Community composition	Development	Bacteria	(Johnston and Rolff, 2015)
Domestic	Perturbation	Bird	Toxicology	Antibiotic	N/A	N/A	N/A	Bacteria	(Jozefiak *et al.*, 2011)
Domestic	Perturbation	Bird	Diet	Drugs	Community composition	Production	N/A	Bacteria	(Józefiak *et al.*, 2013)
Wild	Observation	Invertebrate	Antibiotic	Environment	N/A	N/A	N/A	Bacteria	(Jung *et al.*, 2014)
Domestic	Perturbation	Bird	Age	Infectious disease	Immunity	N/A	N/A	Bacteria	(Juricova *et al.*, 2013)
Model	Perturbation	Mammal	Antibiotic	Drugs	N/A	N/A	N/A	Bacteria	(Kang *et al.*, 2014a)
Model	Perturbation	Mammal	Diet	Exercise	Behaviour	Biomarker	N/A	Bacteria	(Kang *et al.*, 2014b)
Model	Perturbation	Mammal	Diet	Probiotic	Growth	N/A	N/A	Bacteria	(Karlsson *et al.*, 2011)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Kasaikina *et al.*, 2011)
Wild	Observation	Fish	Interspecific comparison	Diet	N/A	N/A	N/A	Bacteria	(Kashinskaya *et al.*, 2014)

Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Kasiraj *et al.*, 2016)
Wild	Observation	Reptile	Community composition	Interspecific comparison	N/A	N/A	N/A	Bacteria	(Keenan *et al.*, 2013)
Wild	Observation	Reptile	Community composition	Interspecific comparison	N/A	N/A	N/A	Bacteria & funghi	(Keene *et al.*, 2014)
Model	Perturbation	Mammal	Prebiotic	Diet	N/A	N/A	N/A	Bacteria	(Ketabi *et al.*, 2011)
Domestic	Perturbation	Bird	Diet	Immunity	Production	N/A	N/A	Bacteria	(Khalaji *et al.*, 2011)
Domestic	Perturbation	Bird	Diet	Antibiotic	Immunity	Production	N/A	Bacteria	(Khan *et al.*, 2012)
Domestic	Perturbation	Non-insect invertebrate	Diet	Production	N/A	N/A	N/A	Bacteria	(Khempaka *et al.*, 2011)
Model	Perturbation	Mammal	Immunity	Infectious disease	N/A	N/A	N/A	Bacteria	(Khosravi *et al.*, 2014)
Model	Perturbation	Mammal	Infectious disease	Hormones	N/A	N/A	N/A	Bacteria	(Khosravi *et al.*, 2016)
Domestic	Observation	Fish	Domesticatio n	N/A	N/A	N/A	N/A	Bacteria	(Kim and Kim, 2013)
Domestic	Perturbation	Mammal	Antibiotic	N/A	N/A	N/A	N/A	Bacteria	(Kim *et al.*, 2012a)
Domestic	Perturbation	Bird	Production	Probiotic	Antibiotic	N/A	N/A	Bacteria	(Kim *et al.*, 2012b)
Domestic	Perturbation	Bird	Diet	Infectious disease	Genotype	N/A	N/A	Bacteria	(Kim *et al.*, 2015)
Domestic	Perturbation	Mammal	Antibiotic	Production	N/A	N/A	N/A	Bacteria	(Kim *et al.*, 2016)
Wild	Observation	Non-insect invertebrate	Environment	N/A	N/A	N/A	N/A	Bacteria & archaea	(King *et al.*, 2012)
Model	Perturbation	Mammal	Toxicology	Genotype	Immunity	N/A	N/A	Bacteria	(Kish *et al.*, 2013)
Domestic	Perturbation	Mammal	Interspecific comparison	N/A	N/A	N/A	N/A	Bacteria, archaea, funghi & protozoa	(Kittelmann *et al.*, 2013)
Model	Perturbation	Mammal	Non-	Antibiotic	Genotype	Immunity	N/A	Bacteria	(Klimesova *et al.*, 2013)

			infectious disease						
Wild	Perturbation	Insect	Diet	N/A	N/A	N/A	N/A	Bacteria	(Knapp *et al.*, 2010)
Domestic	Perturbation	Bird	Diet	Prebiotic	Production	N/A	N/A	Bacteria	(Koc *et al.*, 2010)
Wild	Observation	Insect	Community composition	Interspecific comparison	Infectious disease	N/A	N/A	Bacteria	(Koch and Schmid-Hempel, 2011)
Model	Observation	Insect	Bacterial transplant	Genotype	Infectious disease	N/A	N/A	Bacteria	(Koch and Schmid-Hempel, 2012)
Model	Perturbation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Koh *et al.*, 2015)
Domestic	Perturbation	Fish	Antibiotic	Diet	Production	N/A	N/A	Bacteria	(Koh *et al.*, 2016)
Wild	Observation	Amphibian	Age	Diet	N/A	N/A	N/A	Bacteria	(Kohl *et al.*, 2013)
Wild	Perturbation	Mammal	Toxicology	Diet	N/A	N/A	N/A	Bacteria	(Kohl *et al.*, 2016)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria & archaea	(Kong *et al.*, 2010)
Wild	Observation	Mammal	Community composition	Diet	N/A	N/A	N/A	Bacteria	(Kong *et al.*, 2014a)
Domestic	Perturbation	Mammal	Diet	Prebiotic	N/A	N/A	N/A	Bacteria	(Kong *et al.*, 2014b)
Domestic	Perturbation	Mammal	Genotype	Diet	Production	Immunity	N/A	Bacteria	(Kongsted *et al.*, 2015)
Domestic	Observation	Bird	Genotype	Production	N/A	N/A	N/A	Bacteria	(Konsak *et al.*, 2013)
Model	Observation	Mammal	Interspecific comparison	Genotype	Domesticatio n	N/A	N/A	Bacteria	(Kreisinger *et al.*, 2014)
Model	Perturbation	Mammal	Drugs	Non-infectious disease	Immunity	Genotype	N/A	Bacteria	(Kurata *et al.*, 2015)
Domestic	Perturbation	Bird	Antibiotic	N/A	N/A	N/A	N/A	Bacteria	(La-ongkhum *et al.*, 2011)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Lacombe *et al.*, 2013)
Model	Perturbation	Mammal	Antibiotic	Immunity	Non-	Probiotic	N/A	Bacteria,	(Lam *et al.*, 2012a)

					infectious disease			archaea & funghi	
Model	Perturbation	Mammal	Non-infectious disease	Immunity	Diet	N/A	N/A	Bacteria	(Lam *et al.*, 2012b)
Model	Perturbation	Mammal	Exercise	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Lambert *et al.*, 2015)
Model	Observation	Fish	Methods	N/A	N/A	N/A	N/A	Bacteria	(Larsen *et al.*, 2015)
Domestic	Perturbation	Mammal	Immunity	N/A	N/A	N/A	N/A	Bacteria	(Laycock *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	Environment	N/A	N/A	N/A	Bacteria	(Le Floc'h *et al.*, 2014)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Le Roy *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Lecomte *et al.*, 2015)
Domestic	Perturbation	Mammal	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Lee *et al.*, 2009)
Domestic	Observation	Mammal	Age	Interspecific comparison	N/A	N/A	N/A	Bacteria & archaea	(Lee *et al.*, 2012)
Domestic	Perturbation	Bird	Antibiotic	Probiotic	Production	N/A	N/A	Bacteria	(Lei *et al.*, 2014)
Model	Perturbation	Insect	Diet	Behaviour	N/A	N/A	N/A	Bacteria	(Lewis *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	Production	Immunity	N/A	N/A	Bacteria	(Li and Kim, 2014)
Model	Perturbation	Mammal	Diet	Organ transplant	Non-infectious disease	N/A	N/A	Bacteria	(Li *et al.*, 2011)
Domestic	Perturbation	Mammal	Diet	Age	Community composition	N/A	N/A	Bacteria	(Li *et al.*, 2012a)
Domestic	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2012b)

Domestic	Observation	Fish	Interspecific comparison	N/A	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2012c)
Domestic	Perturbation	Mammal	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2012d)
Model	Perturbation	Mammal	Drugs	Immunity	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2013a)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2013b)
Domestic	Perturbation	Fish	Production	Genotype	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2013c)
Domestic	Observation	Fish	Interspecific comparison	Community composition	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2014)
Wild	Observation	Insect	Community composition	Age	N/A	N/A	N/A	Bacteria & archaea	(Li *et al.*, 2016a)
Wild	Observation	Mammal	Environment	N/A	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2016b)
Domestic	Perturbation	Bird	Probiotic	Production	N/A	N/A	N/A	Bacteria	(Li *et al.*, 2016c)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Lillis *et al.*, 2011)
Wild	Observation	Insect	Environment	Interspecific comparison	N/A	N/A	N/A	Bacteria	(Lim *et al.*, 2015)
Model	Perturbation	Mammal	Drugs	Toxicology	N/A	N/A	N/A	Bacteria	(Lin *et al.*, 2012)
Wild	Observation	Mammal	Genotype	Environment	N/A	N/A	N/A	Bacteria	(Linnenbrink *et al.*, 2013)
Domestic	Perturbation	Non-insect invertebrate	Probiotic	N/A	N/A	N/A	N/A	Bacteria	(Liu *et al.*, 2010)
Domestic	Observation	Non-insect invertebrate	Community composition	Methods	N/A	N/A	N/A	Bacteria	(Liu *et al.*, 2011a)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria	(Liu *et al.*, 2011b)
Domestic	Perturbation	Fish	Antibiotic	Infectious disease	N/A	N/A	N/A	Bacteria	(Liu *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria, archaea, funghi &	(Liu *et al.*, 2014a)

								protozoa	
Domestic	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria & archaea	(Liu *et al.*, 2014b)
Model	Perturbation	Bird	Genotype	Metabolism	Diet	Immunity	N/A	Bacteria	(Liu *et al.*, 2015)
Wild	Observation	Fish	Diet	Metabolism	N/A	N/A	N/A	Bacteria & archaea	(Liu *et al.*, 2016a)
Model	Perturbation	Mammal	Surgical procedure	Genotype	N/A	N/A	N/A	Bacteria	(Liu *et al.*, 2016b)
Model	Perturbation	Insect	Diet	Genotype	Behaviour	N/A	N/A	Bacteria	(Lizé *et al.*, 2013)
Domestic	Perturbation	Fish	Probiotic	Age	Production	N/A	N/A	Bacteria	(Lobo *et al.*, 2014)
Wild	Observation	Insect	Community composition	Environment	N/A	N/A	N/A	Bacteria & funghi	(Long *et al.*, 2010)
Domestic	Perturbation	Mammal	Antibiotic	Community composition	Infectious disease	N/A	N/A	Bacteria	(Looft *et al.*, 2014a)
Domestic	Perturbation	Mammal	Antibiotic	N/A	N/A	N/A	N/A	Bacteria	(Looft *et al.*, 2014b)
Wild	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria, archaea, funghi & viruses	(Lu *et al.*, 2012)
Wild	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Lu *et al.*, 2014a)
Model	Perturbation	Mammal	Toxicology	Genotype	N/A	N/A	N/A	Bacteria	(Lu *et al.*, 2014b)
Model	Perturbation	Mammal	Immunity	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Lundberg *et al.*, 2012)
Model	Perturbation	Mammal	Non-infectious disease	Diet	N/A	N/A	N/A	Bacteria	(MacFarlane *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria	(Magistrelli *et al.*, 2016)

Domestic	Perturbation	Mammal	Diet	Immunity	Community composition	N/A	N/A	Bacteria	(Malmuthuge *et al.*, 2013)
Wild	Observation	Insect	Environment	N/A	N/A	N/A	N/A	Bacteria	(Manjula *et al.*, 2016)
Domestic	Perturbation	Mammal	Immunity	Infectious disease	N/A	N/A	N/A	Bacteria	(Mann *et al.*, 2014a)
Domestic	Perturbation	Mammal	Diet	Age	Community composition	N/A	N/A	Bacteria	(Mann *et al.*, 2014b)
Domestic	Observation	Fish	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Mansfield *et al.*, 2010)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Mao *et al.*, 2013)
Domestic	Perturbation	Mammal	Metabolism	Diet	N/A	N/A	N/A	Bacteria, archaea & funghi	(Mao *et al.*, 2016)
Model	Perturbation	Mammal	Probiotic	Non-infectious disease	Stem cells	N/A	N/A	Bacteria	(Mar *et al.*, 2014)
Domestic	Perturbation	Mammal	Probiotic	Genotype	Production	Immunity	N/A	Bacteria	(Maragkoudakis *et al.*, 2010)
Model	Perturbation	Mammal	Metabolism	N/A	N/A	N/A	N/A	Bacteria	(Mardinoglu *et al.*, 2015)
Model	Observation	Mammal	Bacterial transplant	Immunity	Genotype	N/A	N/A	Bacteria	(Markle *et al.*, 2013
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Marungruang *et al.*, 2016)
Model	Perturbation	Amphibian	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Mashoof *et al.*, 2013)
Domestic	Observation	Bird	Community composition	N/A	N/A	N/A	N/A	Bacteria & archaea	(Matsui *et al.*, 2010)
Wild	Perturbation	Insect	Immunity	Infectious disease	N/A	N/A	N/A	Bacteria	(Matsumoto *et al.*, 2014)
Wild	Observation	Mammal	Temporal	Environment	N/A	N/A	N/A	Bacteria & protozoa	(Maurice *et al.*, 2015)
Model	Perturbation	Mammal	Diet	Metabolism	Growth	N/A	N/A	Bacteria	(McAllan *et al.*, 2014)

Domestic	Perturbation	Mammal	Genotype	N/A	N/A	N/A	N/A	Bacteria	(McCann *et al.*, 2014)
Wild	Perturbation	Fish	Diet	N/A	N/A	N/A	N/A	Bacteria	(McDonald *et al.*, 2012)
Wild	Observation	Insect	Community composition	Age	N/A	N/A	N/A	Bacteria & funghi	(McFrederick *et al.*, 2014)
Model	Observation	Mammal	Genotype	Immunity	N/A	N/A	N/A	Bacteria	(McKnite *et al.*, 2012)
Model	Perturbation	Mammal	Gut-brain axis	N/A	N/A	N/A	N/A	Bacteria	(McVey Neufeld *et al.*, 2015)
Domestic	Perturbation	Bird	Genotype	N/A	N/A	N/A	N/A	Bacteria	(Meng *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Hormones	N/A	N/A	N/A	Bacteria & archaea	(Menon *et al.*, 2013)
Domestic	Perturbation	Fish	Probiotic	Production	Immunity	N/A	N/A	Bacteria	(Merrifield *et al.*, 2010)
Domestic	Perturbation	Fish	Diet	Production	Immunity	N/A	N/A	Bacteria	(Merrifield *et al.*, 2011)
Domestic	Perturbation	Mammal	Genotype	Diet	N/A	N/A	N/A	Bacteria	(Messori *et al.*, 2013)
Wild	Observation	Mammal	Interspecific comparison	Environment	Phylogeny	N/A	N/A	Bacteria	(Moeller *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Moen *et al.*, 2016)
Domestic	Perturbation	Bird	Probiotic	Production	N/A	N/A	N/A	Bacteria	(Mohammadi Gheisar *et al.*, 2016a)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Mohammadi Gheisar *et al.*, 2016b)
Domestic	Perturbation	Mammal	Diet	Community composition	N/A	N/A	N/A	Bacteria, archaea & protozoa	(Mohammadzadeh *et al.*, 2014)
Domestic	Perturbation	Fish	Diet	Probiotic	Production	N/A	N/A	Bacteria	(Mohapatra *et al.*, 2012)
Domestic	Observation	Bird	Community composition	Function	Age	N/A	N/A	Bacteria	(Mohd Shaufi *et al.*, 2015)
Wild	Observation	Insect	Genotype	Environment	Community composition	N/A	N/A	Bacteria, archaea &	(Moran *et al.*, 2012)

								funghi	
Domestic	Perturbation	Mammal	Diet	Domestication	Immunity	N/A	N/A	Bacteria	(Morán *et al.*, 2012)
Domestic	Perturbation	Mammal	Probiotic	Vertical transmission	N/A	N/A	N/A	Bacteria	(Mori *et al.*, 2011)
Model	Perturbation	Mammal	Antibiotic	Non-infectious disease	Age	N/A	N/A	Bacteria	(Mozeš *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Mujico *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Murphy *et al.*, 2012)
Model	Perturbation	Mammal	Antibiotic	Probiotic	Diet	N/A	N/A	Bacteria	(Murphy *et al.*, 2013)
Model	Perturbation	Mammal	Drugs	N/A	N/A	N/A	N/A	Bacteria	(Musch *et al.*, 2013)
Domestic	Observation	Mammal	Production	Community composition	N/A	N/A	N/A	Bacteria	(Myer *et al.*, 2016)
Model	Perturbation	Mammal	Infectious disease	Antibiotic	Immunity	N/A	N/A	Bacteria	(Nagalingam *et al.*, 2013)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria	(Nahavandinejad *et al.*, 2012)
Domestic	Perturbation	Fish	Diet	N/A	N/A	N/A	N/A	Bacteria	(Najdegerami *et al.*, 2012)
Model	Perturbation	Mammal	Infectious disease	Immunity	Genotype	N/A	N/A	Bacteria	(Nakajima *et al.*, 2015)
Domestic	Perturbation	Bird	Probiotic	Production	N/A	N/A	N/A	Bacteria	(Nakphaichit *et al.*, 2011)
Wild	Perturbation	Insect	Immunity	Bacterial transplant	Infectious disease	N/A	N/A	Bacteria	(Näpflin and Schmid-Hempel, 2016)
Model	Perturbation	Fish	Community composition	Antibiotic	N/A	N/A	N/A	Bacteria & archaea	(Narrowe *et al.*, 2015)
Domestic	Observation	Bird	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Nathiya *et al.*, 2012)

Model	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Nava *et al.*, 2011)
Domestic	Perturbation	Fish	Diet	Genotype	N/A	N/A	N/A	Bacteria	(Navarrete *et al.*, 2012)
Model	Perturbation	Mammal	Genotype	Infectious disease	N/A	N/A	N/A	Bacteria	(Nelson *et al.*, 2013a)
Wild	Observation	Mammal	Interspecific comparison	Age	Diet	Domestication	N/A	Bacteria	(Nelson *et al.*, 2013b)
Model	Perturbation	Insect	Community composition	Diet	N/A	N/A	N/A	Bacteria	(Newell and Douglas, 2014)
Domestic	Perturbation	Fish	Community composition	Diet	Metabolism	N/A	N/A	Bacteria & archaea	(Ni *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Noratto *et al.*, 2014)
Domestic	Perturbation	Bird	Environment	Infectious disease	N/A	N/A	N/A	Bacteria	(Nordentoft *et al.*, 2011)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Norouzi *et al.*, 2015)
Model	Perturbation	Mammal	Antibiotic	Behaviour	Gut-brain axis	N/A	N/A	Bacteria	(O'Mahony *et al.*, 2014)
Domestic	Observation	Bird	Community composition	Age	Environment	Immunity	N/A	Bacteria	(Oakley and Kogut, 2016)
Model	Perturbation	Mammal	Probiotic	Genotype	Diet	Immunity	Behaviour	Bacteria	(Ohland *et al.*, 2013)
Domestic	Observation	Mammal	Age	Genotype	N/A	N/A	N/A	Bacteria	(Oikonomou *et al.*, 2013)
Wild	Perturbation	Insect	Infectious disease	Diet	Immunity	N/A	N/A	Bacteria	(Oliveira *et al.*, 2011)
Wild	Observation	Insect	Age	N/A	N/A	N/A	N/A	Bacteria	(Olivier-Espejel *et al.*, 2011)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Omazic *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Omoniyi *et al.*, 2014)
Model	Perturbation	Mammal	Non-	Genotype	Antibiotic	Immunity	N/A	Bacteria	(Ooi *et al.*, 2013)

			infectious disease						
Domestic	Perturbation	Fish	Diet	Prebiotic	Production	N/A	N/A	Bacteria	(Ortiz *et al.*, 2013)
Wild	Observation	Insect	Infectious disease	Interspecific comparison	Community composition	N/A	N/A	Bacteria	(Osei-Poku *et al.*, 2012)
Domestic	Perturbation	Mammal	Infectious disease	Drugs	N/A	N/A	N/A	Bacteria	(Paddock *et al.*, 2014)
Domestic	Observation	Mammal	Interspecific comparison	Genotype	N/A	N/A	N/A	Bacteria	(Pajarillo *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Palmnäs *et al.*, 2014)
Model	Perturbation	Mammal	Genotype	N/A	N/A	N/A	N/A	Bacteria	(Pang *et al.*, 2012a)
Model	Observation	Mammal	Community composition	Age	N/A	N/A	N/A	Bacteria	(Pang *et al.*, 2012b)
Model	Perturbation	Mammal	Age	Vertical transmission	N/A	N/A	N/A	Bacteria	(Pantoja-Feliciano *et al.*, 2013)
Domestic	Perturbation	Mammal	Diet	Metabolism	N/A	N/A	N/A	Bacteria	(Papadomichelakis *et al.*, 2012)
Model	Perturbation	Mammal	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Park *et al.*, 2013)
Domestic	Observation	Mammal	Community composition	Genotype	N/A	N/A	N/A	Bacteria	(Park *et al.*, 2014)
Domestic	Perturbation	Bird	Prebiotic	N/A	N/A	N/A	N/A	Bacteria & archaea	(Park *et al.*, 2016)
Domestic	Perturbation	Mammal	Diet	Vertical transmission	N/A	N/A	N/A	Bacteria	(Paßlack *et al.*, 2015)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Patrone *et al.*, 2012)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Patterson *et al.*, 2014)
Model	Observation	Bird	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Pauwels *et al.*, 2015)

Model	Perturbation	Mammal	Non-infectious disease	Genotype	Community composition	Diet	N/A	Bacteria & archaea	(Pedersen *et al.*, 2013)
Model	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Pédron *et al.*, 2012)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria	(Peinado *et al.*, 2013)
Model	Perturbation	Mammal	Antibiotic	Immunity	N/A	N/A	N/A	Bacteria	(Pélissier *et al.*, 2010)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Peng *et al.*, 2014)
Wild	Observation	Mammal	Age	Antibiotic	N/A	N/A	N/A	Bacteria	(Peng *et al.*, 2016)
Domestic	Perturbation	Bird	Diet	Infectious disease	N/A	N/A	N/A	Bacteria	(Perez *et al.*, 2011)
Model	Perturbation	Insect	Diet	N/A	N/A	N/A	N/A	Bacteria	(Pérez-Cobas *et al.*, 2015)
Domestic	Perturbation	Mammal	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Perkins *et al.*, 2012)
Domestic	Perturbation	Bird	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Perumbakkam *et al.*, 2014)
Model	Perturbation	Mammal	Prebiotic	Infectious disease	N/A	N/A	N/A	Bacteria	(Petersen *et al.*, 2010)
Domestic	Observation	Mammal	Diet	Methods	N/A	N/A	N/A	Bacteria	(Petersson *et al.*, 2010)
Model	Perturbation	Mammal	Exercise	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Petriz *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Genotype	Metabolism	Non-infectious disease	N/A	Bacteria	(Pfalzer *et al.*, 2015)
Domestic	Perturbation	Mammal	Toxicology	N/A	N/A	N/A	N/A	Bacteria & funghi	(Piotrowska *et al.*, 2014)

Domestic	Observation	Bird	Environment	Community composition	N/A	N/A	N/A	Bacteria	(Pissavin *et al.*, 2012)
Domestic	Perturbation	Mammal	Age	Diet	Community composition	N/A	N/A	Bacteria	(Pitta *et al.*, 2014)
Domestic	Observation	Mammal	Non-infectious disease	Metabolism	N/A	N/A	N/A	Bacteria & archaea	(Pitta *et al.*, 2016)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Placha *et al.*, 2013)
Model	Perturbation	Mammal	Infectious disease	Non-infectious disease	N/A	N/A	N/A	Bacteria & archaea	(Plieskatt *et al.*, 2013)
Model	Perturbation	Mammal	Drugs	Toxicology	N/A	N/A	N/A	Bacteria	(Possamai *et al.*, 2015)
Domestic	Perturbation	Bird	Prebiotic	Antibiotic	N/A	N/A	N/A	Bacteria	(Pourabedin *et al.*, 2014)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria	(Pourhossein, 2012)
Domestic	Perturbation	Mammal	Probiotic	N/A	N/A	N/A	N/A	Bacteria	(Præsteng *et al.*, 2013)
Wild	Observation	Insect	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Praet *et al.*, 2016)
Model	Perturbation	Mammal	Non-infectious disease	Diet	N/A	N/A	N/A	Bacteria	(Prajapati *et al.*, 2015)
Domestic	Perturbation	Bird	Diet	Infectious disease	N/A	N/A	N/A	Bacteria	(Prasai *et al.*, 2016)
Domestic	Perturbation	Mammal	Antibiotic	Probiotic	N/A	N/A	N/A	Bacteria	(Puiman *et al.*, 2013)
Model	Perturbation	Mammal	Behaviour	Diet	Immunity	Gut-brain axis	N/A	Bacteria	(Pyndt Jørgensen *et al.*, 2014)
Model	Perturbation	Mammal	Non-infectious disease	Gut-brain axis	Drugs	Antibiotic	N/A	Bacteria	(Pyndt Jørgensen *et al.*, 2015)
Model	Perturbation	Mammal	Diet	Exercise	Metabolism	N/A	N/A	Bacteria &	(Queipo-Ortuño *et al.*, 2013)

								archaea	
Domestic	Perturbation	Fish	Probiotic	N/A	N/A	N/A	N/A	Bacteria	(Ramos *et al.*, 2013)
Wild	Observation	Invertebrate	Drugs	N/A	N/A	N/A	N/A	Bacteria	(Rattray *et al.*, 2010)
Domestic	Perturbation	Reptile	Probiotic	Growth	N/A	N/A	N/A	Bacteria	(Rawski *et al.*, 2016)
Model	Perturbation	Mammal	Genotype	Non-infectious disease	Immunity	N/A	N/A	Bacteria	(Rehaume *et al.*, 2014)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Ren *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Ren *et al.*, 2014a)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Ren *et al.*, 2014b)
Model	Perturbation	Mammal	Biomarker	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Ren *et al.*, 2014c)
Domestic	Perturbation	Mammal	Antibiotic	Community composition	N/A	N/A	N/A	Bacteria	(Reti *et al.*, 2013)
Model	Perturbation	Mammal	Bacterial transplant	Diet	Non-infectious disease	N/A	N/A	Bacteria	(Ridaura *et al.*, 2013)
Wild	Observation	Insect	Community composition	N/A	N/A	N/A	N/A	Bacteria & funghi	(Rinke *et al.*, 2011)
Domestic	Perturbation	Mammal	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Rist *et al.*, 2012)
Model	Perturbation	Mammal	Environment	Diet	N/A	N/A	N/A	Bacteria	(Ritchie *et al.*, 2015)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Rodriguez *et al.*, 2011)
Domestic	Observation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Rodriguez *et al.*, 2015)

Model	Observation	Fish	Community composition	Environment	N/A	N/A	N/A	Bacteria	(Roeselers *et al.*, 2011)
Model	Observation	Mammal	Environment	N/A	N/A	N/A	N/A	Bacteria	(Rogers *et al.*, 2014)
Wild	Perturbation	Mammal	Diet	Community composition	N/A	N/A	N/A	Bacteria & archaea	(Roggenbuck *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Romo-Vaquero *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	Genotype	N/A	N/A	N/A	Bacteria, archaea & funghi	(Rooke *et al.*, 2014)
Wild	Perturbation	Insect	Antibiotic	Interspecific comparison	Temporal	N/A	N/A	Bacteria & protozoa	(Rosengaus *et al.*, 2011)
Domestic	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Rosewarne *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Ross *et al.*, 2013)
Model	Perturbation	Invertebrate	Diet	N/A	N/A	N/A	N/A	Bacteria	(Rudi and Strætkvern, 2012)
Model	Perturbation	Fish	Diet	Immunity	Genotype	N/A	N/A	Bacteria	(Rurangwa *et al.*, 2015)
Wild	Observation	Bird	Interspecific comparison	Infectious disease	N/A	N/A	N/A	Bacteria	(Ryu *et al.*, 2014)
Wild	Observation	Insect	Diet	Interspecific comparison	N/A	N/A	N/A	Bacteria	(Sabree and Moran, 2014)
Model	Perturbation	Mammal	Diet	Prebiotic	N/A	N/A	N/A	Bacteria & archaea	(Saha and Reimer, 2014)
Model	Perturbation	Mammal	Diet	Immunity	N/A	N/A	N/A	Bacteria	(Sahasakul *et al.*, 2012)
Wild	Observation	Fish	Interspecific comparison	N/A	N/A	N/A	N/A	Bacteria	(Sahnouni *et al.*, 2012)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Saki *et al.*, 2011)
Domestic	Perturbation	Bird	Probiotic	Antibiotic	Immunity	Production	N/A	Bacteria	(Salim *et al.*, 2013)

Wild	Observation	Insect and mammal	Phylogeny	Interspecific comparison	N/A	N/A	N/A	Bacteria	(Sanders *et al.*, 2014)
Wild	Observation	Insect	Age	N/A	N/A	N/A	N/A	Bacteria, archaea & funghi	(Santana *et al.*, 2015)
Wild	Observation	Bird	Interspecific comparison	N/A	N/A	N/A	N/A	Bacteria	(Santos *et al.*, 2012)
Wild	Perturbation	Insect	Antibiotic	Community composition	N/A	N/A	N/A	Bacteria	(Sapountzis *et al.*, 2015)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria, funghi & protozoa	(Sarubbi *et al.*, 2014)
Wild	Observation	Insect	Diet	Genotype	Community composition	N/A	N/A	Bacteria	(Schauer *et al.*, 2014)
Model	Perturbation	Mammal	Non-infectious disease	Diet	N/A	N/A	N/A	Bacteria	(Schéle *et al.*, 2013)
Domestic	Perturbation	Mammal	Antibiotic	Domestication	Development	N/A	N/A	Bacteria	(Schokker *et al.*, 2014)
Domestic	Perturbation	Mammal	Antibiotic	Environment	N/A	N/A	N/A	Bacteria	(Schokker *et al.*, 2015)
Domestic	Observation	Mammal	Immunity	Community composition	N/A	N/A	N/A	Bacteria	(Schroedl *et al.*, 2014)
Wild	Observation	Mammal	Community composition	Interspecific comparison	N/A	N/A	N/A	Bacteria	(Schwab and Gänzle, 2011)
Domestic	Perturbation	Bird	Immunity	Antibiotic	Infectious disease	N/A	N/A	Bacteria	(Scupham *et al.*, 2010)
Model	Perturbation	Mammal	Infectious disease	Genotype	Drugs	N/A	N/A	Bacteria	(Seekatz *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Growth	Age	N/A	N/A	Bacteria	(Šefčíková *et al.*, 2011)
Model	Perturbation	Fish	Metabolism	N/A	N/A	N/A	N/A	Bacteria	(Semova *et al.*, 2012)

Model	Perturbation	Mammal	Non-infectious disease	Immunity	Diet	Prebiotic	N/A	Bacteria	(Serino *et al.*, 2011)
Wild	Observation	Insect	Community composition	Age	N/A	N/A	N/A	Bacteria, archaea & funghi	(Shao *et al.*, 2014)
Wild	Observation	Insect	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Sharma *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria & archaea	(Shaw *et al.*, 2016)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Shen *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Singh and Singh, 2013)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria & protozoa	(Singh *et al.*, 2011)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria, archaea & viruses	(Singh *et al.*, 2014)
Model	Observation	Mammal	Development	Immunity	N/A	N/A	N/A	Bacteria	(Sjögren *et al.*, 2012)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Skoufos *et al.*, 2016)
Domestic	Observation	Mammal	Age	N/A	N/A	N/A	N/A	Bacteria	(Slifierz *et al.*, 2015)
Model	Perturbation	Mammal	Prebiotic	Production	Community composition	N/A	N/A	Bacteria	(Śliżewska *et al.*, 2015)
Model	Perturbation	Mammal	Genotype	N/A	N/A	N/A	N/A	Bacteria	(Smith *et al.*, 2012)
Wild	Observation	Mammal	Age	N/A	N/A	N/A	N/A	Bacteria	(Smith *et al.*, 2013)
Wild	Observation	Fish	Interspecific comparison	Community composition	Diet	N/A	N/A	Bacteria & archaea	(Smriga *et al.*, 2010)
Model	Perturbation	Mammal	Non-infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Sommer *et al.*, 2014)

Wild	Observation	Mammal	Metabolism	Temporal	N/A	N/A	N/A	Bacteria	(Sommer *et al.*, 2016)
Model	Perturbation	Mammal	Diet	Non-infectious disease	Immunity	N/A	N/A	Bacteria	(Sonoyama *et al.*, 2010)
Domestic	Perturbation	Bird	Production	Genotype	N/A	N/A	N/A	Bacteria	(Stanley *et al.*, 2012)
Domestic	Observation	Bird	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Stanley *et al.*, 2015)
Domestic	Observation	Bird	Metabolism	N/A	N/A	N/A	N/A	Bacteria	(Stanley *et al.*, 2016)
Domestic	Observation	Fish	Genotype	Community composition	N/A	N/A	N/A	Bacteria	(Star *et al.*, 2013)
Domestic	Perturbation	Mammal	Toxicology	N/A	N/A	N/A	N/A	Bacteria	(Starke *et al.*, 2014)
Model	Observation	Mammal	Genotype	N/A	N/A	N/A	N/A	Bacteria	(Staubach *et al.*, 2012)
Model	Observation	Fish	Development	Community composition	Diet	Environment	N/A	Bacteria	(Stephens *et al.*, 2016)
Wild	Observation	Mammal	Temporal	Age	Genotype	N/A	N/A	Bacteria	(Stevenson *et al.*, 2014a)
Wild	Observation	Mammal	Community composition	Temporal	N/A	N/A	N/A	Bacteria & archaea	(Stevenson *et al.*, 2014b)
Model	Observation	Insect	Vertical transmission	Diet	N/A	N/A	N/A	Bacteria	(Sudakaran *et al.*, 2012)
Domestic	Perturbation	Fish	Probiotic	N/A	N/A	N/A	N/A	Bacteria	(Sun *et al.*, 2012a)
Domestic	Perturbation	Fish	Probiotic	N/A	N/A	N/A	N/A	Bacteria	(Sun *et al.*, 2012b)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Sun *et al.*, 2016)
Model	Perturbation	Mammal	Antibiotic	N/A	N/A	N/A	N/A	Bacteria	(Sze *et al.*, 2014)
Model	Perturbation	Mammal	Age	Diet	N/A	N/A	N/A	Bacteria	(Tachon *et al.*, 2013)
Domestic	Perturbation	Bird	Environment	Production	N/A	N/A	N/A	Bacteria	(Taherparvar *et al.*, 2016)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Tamura *et al.*, 2012a)
Model	Perturbation	Mammal	Prebiotic	Diet	N/A	N/A	N/A	Bacteria	(Tamura *et al.*, 2012b)

Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Tamura *et al.*, 2013)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Tancharoenrat *et al.*, 2014)
Wild	Observation	Insect	Community composition	Interspecific comparison	Diet	N/A	N/A	Bacteria & archaea	(Tang *et al.*, 2012a)
Domestic	Observation	Mammal	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Tang *et al.*, 2012b)
Domestic	Observation	Mammal	Age	Immunity	N/A	N/A	N/A	Bacteria	(Tao *et al.*, 2015)
Domestic	Perturbation	Fish	Probiotic	Antibiotic	Immunity	N/A	N/A	Bacteria	(Tapia-Paniagua *et al.*, 2015)
Wild	Observation	Insect	Age	Development	Community composition	N/A	N/A	Bacteria	(Tarpy *et al.*, 2015)
Domestic	Perturbation	Mammal	Function	Metabolism	Community composition	N/A	N/A	Bacteria	(Taxis *et al.*, 2015)
Domestic	Perturbation	Bird	Diet	Development	N/A	N/A	N/A	Bacteria	(Tellez *et al.*, 2014)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Teng *et al.*, 2016)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Terán-Ventura *et al.*, 2014)
Wild	Observation	Non-insect invertebrate	Diet	Age	N/A	N/A	N/A	Bacteria	(Tetlock *et al.*, 2012)
Model	Perturbation	Insect	Antibiotic	Growth	N/A	N/A	N/A	Bacteria	(Thakur *et al.*, 2016)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Thoetkiattikul *et al.*, 2013)
Domestic	Perturbation	Bird	Antibiotic	N/A	N/A	N/A	N/A	Bacteria	(Tillman *et al.*, 2011)
Domestic	Perturbation	Bird	Antibiotic	Community composition	Age	Production	N/A	Bacteria	(Torok *et al.*, 2011a)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Torok *et al.*, 2011b)
Domestic	Observation	Bird	Genotype	Environment	Diet	Production	N/A	Bacteria	(Torok *et al.*, 2013)

Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria, archaea, funghi & protozoa	(Torok *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	Antibiotic	Immunity	N/A	N/A	Bacteria	(Tran *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Tsai *et al.*, 2015)
Wild	Observation	Mammal	Age	N/A	N/A	N/A	N/A	Bacteria & funghi	(Tun *et al.*, 2014)
Domestic	Perturbation	Mammal	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Twardziok *et al.*, 2014)
Wild	Observation	Non-insect invertebrate	Phylogeny	Environment	Interspecific comparison	N/A	N/A	Bacteria	(Tzeng *et al.*, 2015)
Model	Observation	Mammal	Genotype	Immunity	Domestication	N/A	N/A	Bacteria	(Ubeda *et al.*, 2012)
Domestic	Perturbation	Mammal	Antibiotic	Age	Production	N/A	N/A	Bacteria & archaea	(Unno *et al.*, 2015)
Domestic	Perturbation	Mammal	Probiotic	N/A	N/A	N/A	N/A	Bacteria	(Upadrasta *et al.*, 2013)
Domestic	Perturbation	Mammal	Probiotic	Production	Diet	N/A	N/A	Bacteria & protozoa	(Ushakova *et al.*, 2013)
Model	Perturbation	Mammal	Genotype	Diet	Environment	Metabolism	N/A	Bacteria	(Ussar *et al.*, 2015)
Domestic	Observation	Mammal	Environment	Gut-brain axis	N/A	N/A	N/A	Bacteria	(Uyeno *et al.*, 2010)
Model	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Uyeno *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	Probiotic	Immunity	N/A	N/A	Bacteria	(Valdovska *et al.*, 2014)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(van der Hoeven-Hangoor *et al.*, 2013)
Wild	Observation	Bird	Age	N/A	N/A	N/A	N/A	Bacteria	(van Dongen *et al.*, 2013)
Domestic	Perturbation	Bird	Interspecific	Diet	N/A	N/A	N/A	Bacteria	(Vasaï *et al.*, 2014a)

			comparison						
Domestic	Perturbation	Bird	Probiotic	Diet	N/A	N/A	N/A	Bacteria	(Vasaï *et al.*, 2014b)
Model	Perturbation	Mammal	Drugs	Immunity	N/A	N/A	N/A	Bacteria & archaea	(Verma *et al.*, 2014)
Model	Observation	Mammal	Genotype	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Vestergaard *et al.*, 2015)
Domestic	Perturbation	Mammal	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Vhile *et al.*, 2012)
Domestic	Perturbation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Videnska *et al.*, 2013)
Wild	Observation	Insect	Community composition	N/A	N/A	N/A	N/A	Bacteria & archaea	(Waite *et al.*, 2015)
Model	Perturbation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Walk *et al.*, 2010)
Domestic	Perturbation	Mammal	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Walsh *et al.*, 2012)
Domestic	Perturbation	Mammal	Prebiotic	N/A	N/A	N/A	N/A	Bacteria	(Walsh *et al.*, 2013)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria	(Walugembe *et al.*, 2015)
Domestic	Perturbation	Bird	Diet	Immunity	Production	Antibiotic	N/A	Bacteria	(Wang *et al.*, 2010a)
Domestic	Perturbation	Mammal	Prebiotic	Immunity	Production	N/A	N/A	Bacteria	(Wang *et al.*, 2010b)
Wild	Observation	Insect	Age	Diet	Community composition	N/A	N/A	Bacteria	(Wang *et al.*, 2011)
Domestic	Perturbation	Bird	Diet	N/A	N/A	N/A	N/A	Bacteria	(Wang *et al.*, 2012)
Model	Perturbation	Mammal	Probiotic	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Wang *et al.*, 2013a)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Wang *et al.*, 2013b)

Model	Perturbation	Mammal	Probiotic	Growth	N/A	N/A	N/A	Bacteria	(Wang *et al.*, 2015)
Domestic	Perturbation	Bird	Environment	Age	Horizontal transmission	N/A	N/A	Bacteria	(Wang *et al.*, 2016)
Model	Perturbation	Insect	Diet	Infectious disease	N/A	N/A	N/A	Bacteria	(Wayland *et al.*, 2014)
Domestic	Observation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Weese *et al.*, 2015)
Model	Perturbation	Mammal	Non-infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Wei *et al.*, 2010)
Domestic	Observation	Bird	Interspecific comparison	N/A	N/A	N/A	N/A	Bacteria & archaea	(Wei *et al.*, 2013)
Model	Perturbation	Mammal	Immunity	Antibiotic	Genotype	N/A	N/A	Bacteria	(Williams *et al.*, 2015)
Model	Perturbation	Mammal	Antibiotic	Gut-brain axis	Non-infectious disease	N/A	N/A	Bacteria	(Winek *et al.*, 2016)
Domestic	Perturbation	Bird	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Witzig *et al.*, 2015)
Domestic	Perturbation	Fish	Environment	Diet	Community composition	N/A	N/A	Bacteria	(Wong *et al.*, 2013)
Model	Perturbation	Insect	Diet	Growth	N/A	N/A	N/A	Bacteria	(Wong *et al.*, 2014)
Wild	Perturbation	Insect	Horizontal transmission	Probiotic	N/A	N/A	N/A	Bacteria	(Woodbury *et al.*, 2013)
Domestic	Observation	Fish	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Wu *et al.*, 2010)
Domestic	Observation	Fish	Community composition	Diet	Environment	N/A	N/A	Bacteria	(Wu *et al.*, 2013)
Domestic	Perturbation	Bird	Diet	Toxicology	N/A	N/A	N/A	Bacteria	(Wu *et al.*, 2014a)
Domestic	Perturbation	Bird	Diet	Infectious	N/A	N/A	N/A	Bacteria	(Wu *et al.*, 2014b)

				disease					
Wild	Observation	Bird	Interspecific comparison	Domestication	N/A	N/A	N/A	Bacteria	(Xenoulis *et al.*, 2010)
Model	Perturbation	Mammal	Organ transplant	Non-infectious disease	Immunity	N/A	N/A	Bacteria	(Xie *et al.*, 2011)
Model	Perturbation	Mammal	Probiotic	Antibiotic	Immunity	Organ transplant	N/A	Bacteria	(Xie *et al.*, 2014)
Model	Perturbation	Mammal	Drugs	Probiotic	Non-infectious disease	N/A	N/A	Bacteria	(Xie *et al.*, 2016)
Model	Perturbation	Mammal	Antibiotic	N/A	N/A	N/A	N/A	Bacteria	(Xin-Li *et al.*, 2012)
Model	Perturbation	Mammal	Drugs	Immunity	N/A	N/A	N/A	Bacteria	(Xu and Zhang, 2015)
Domestic	Perturbation	Mammal	Immunity	Infectious disease	N/A	N/A	N/A	Bacteria	(Xu *et al.*, 2014a)
Domestic	Perturbation	Mammal	Domestication	Drugs	N/A	N/A	N/A	Bacteria	(Xu *et al.*, 2014b)
Model	Perturbation	Mammal	Immunity	N/A	N/A	N/A	N/A	Bacteria	(Xue *et al.*, 2014)
Wild	Observation	Mammal	Temporal	Community composition	Phylogeny	N/A	N/A	Bacteria	(Xue *et al.*, 2015)
Domestic	Observation	Non-insect invertebrate	Community composition	Growth	N/A	N/A	N/A	Bacteria & archaea	(Yamazaki *et al.*, 2016)
Domestic	Perturbation	Fish	Probiotic	Community composition	N/A	N/A	N/A	Bacteria	(Yang *et al.*, 2012)
Model	Perturbation	Mammal	Environment	Infectious disease	N/A	N/A	N/A	Bacteria	(Yang *et al.*, 2013)
Domestic	Perturbation	Fish	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Yang *et al.*, 2014a)
Domestic	Observation	Mammal	Genotype	N/A	N/A	N/A	N/A	Bacteria & archaea	(Yang *et al.*, 2014b)

Domestic	Perturbation	Non-insect invertebrate	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Yang *et al.*, 2015)
Domestic	Observation	Fish	Interspecific comparison	Temporal	Environment	Diet	N/A	Bacteria & archaea	(Ye *et al.*, 2014)
Domestic	Observation	Bird	Bacterial transplant	Age	N/A	N/A	N/A	Bacteria	(Yin *et al.*, 2010)
Model	Perturbation	Mammal	Diet	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Yin *et al.*, 2013)
Model	Perturbation	Mammal	Probiotic	N/A	N/A	N/A	N/A	Bacteria	(Yin *et al.*, 2014)
Model	Perturbation	Mammal	Probiotic	Immunity	N/A	N/A	N/A	Bacteria	(Yoda *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria	(Yu *et al.*, 2016a)
Model	Perturbation	Mammal	Probiotic	Prebiotic	N/A	N/A	N/A	Bacteria	(Yu *et al.*, 2016b)
Model	Perturbation	Mammal	Immunity	Infectious disease	N/A	N/A	N/A	Bacteria & funghi	(Zaiss *et al.*, 2015)
Domestic	Perturbation	Fish	Diet	Temporal	Production	N/A	N/A	Bacteria	(Zarkasi *et al.*, 2016)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Zdunczyk *et al.*, 2014)
Domestic	Perturbation	Mammal	Diet	N/A	N/A	N/A	N/A	Bacteria	(Zened *et al.*, 2013)
Model	Perturbation	Mammal	Domestication	N/A	N/A	N/A	N/A	Bacteria	(Zeng *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	Community composition	N/A	N/A	N/A	Bacteria	(Zentek *et al.*, 2012)
Domestic	Perturbation	Mammal	Diet	Production	Community composition	N/A	N/A	Bacteria	(Zentek *et al.*, 2013a)
Domestic	Perturbation	Mammal	Diet	Immunity	Production	N/A	N/A	Bacteria	(Zentek *et al.*, 2013b)
Model	Perturbation	Mammal	Non-infectious disease	Immunity	N/A	N/A	N/A	Bacteria	(Zhan *et al.*, 2013)
Domestic	Perturbation	Bird	Probiotic	Production	N/A	N/A	N/A	Bacteria	(Zhang and Kim, 2014)

Model	Perturbation	Mammal	Diet	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Zhang *et al.*, 2012)
Domestic	Perturbation	Bird	Diet	Environment	Production	Immunity	N/A	Bacteria	(Zhang *et al.*, 2013a)
Model	Perturbation	Mammal	Antibiotic	Probiotic	N/A	N/A	N/A	Bacteria	(Zhang *et al.*, 2013b)
Domestic	Perturbation	Bird	Probiotic	Production	Antibiotic	N/A	N/A	Bacteria	(Zhang *et al.*, 2013c)
Domestic	Perturbation	Mammal	Diet	Production	N/A	N/A	N/A	Bacteria	(Zhang *et al.*, 2014a)
Wild	Observation	Insect	Community composition	N/A	N/A	N/A	N/A	Bacteria	(Zhang *et al.*, 2014b)
Model	Perturbation	Mammal	Antibiotic	Metabolism	N/A	N/A	N/A	Bacteria	(Zhang *et al.*, 2014c)
Model	Perturbation	Mammal	Probiotic	Non-infectious disease	N/A	N/A	N/A	Bacteria	(Zhang *et al.*, 2015)
Domestic	Perturbation	Mammal	Antibiotic	Probiotic	N/A	N/A	N/A	Bacteria	(Zhao and Kim, 2015)
Domestic	Perturbation	Bird	Genotype	N/A	N/A	N/A	N/A	Bacteria & archaea	(Zhao *et al.*, 2013a)
Domestic	Perturbation	Bird	Probiotic	Production	N/A	N/A	N/A	Bacteria	(Zhao *et al.*, 2013b)
Domestic	Perturbation	Bird	Diet	Production	N/A	N/A	N/A	Bacteria	(Zhao *et al.*, 2013c)
Domestic	Perturbation	Mammal	Diet	Production	Probiotic	Immunity	N/A	Bacteria	(Zhao *et al.*, 2015a)
Domestic	Observation	Mammal	Age	Community composition	Metabolism	N/A	N/A	Bacteria	(Zhao *et al.*, 2015b)
Domestic	Perturbation	Fish	Genotype	Antibiotic	Prebiotic	Production	N/A	Bacteria	(Zhou *et al.*, 2011)
Model	Perturbation	Mammal	Non-infectious disease	Prebiotic	N/A	N/A	N/A	Bacteria	(Zhou *et al.*, 2013)
Model	Perturbation	Mammal	Diet	Growth	Immunity	N/A	N/A	Bacteria	(Zhou *et al.*, 2015)
Model	Perturbation	Mammal	Non-	N/A	N/A	N/A	N/A	Bacteria	(Zhu *et al.*, 2014)

			infectious disease						
Model	Perturbation	Mammal	Diet	Age	N/A	N/A	N/A	Bacteria	(Zhu *et al.*, 2015)
Domestic	Observation	Mammal	Infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Zinicola *et al.*, 2015)

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Appendix A.2: Supplementary figure for data presented in Chapters 3, 4, 5 & 6

16S Amplicon PCR Forward Primer (341F) =

5' TCGTCGGCAGCGTCAGATGTGTATAAGCCTACGGGNGGCWGCAG **3'**

16S Amplicon PCR Reverse Primer (805R) =

5' GTCTCGTGGGCTCGGAGATGTGTATAAGACTACHVGGGTATCTAATCC **3'**

Figure A.2.1: The nucleotide sequences, including degenerate nucleotides, of the forward and reverse primers, used in PCR reactions to target 16S rRNA in samples. Nucleotides in grey indicate the Illumina adaptor sequences.

Appendix A.3: Supplementary tables of data presented in Chapter 3

Statistical outputs of analyses to test for OTUs that significantly differed in abundance between pre- and post-treatment individuals in an anthelmintic and a control group, for microbiota of the whole gut (three gut sections combined), small intestine, caecum, colon and faeces. OTUs were grouped by microbial phylum and class. Briefly, DESeq was used to identify significantly different (p <0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing pre- and post-treatment mice. Below are the tables resulting from these analyses.

Note: Abundances of OTUs in the small intestine and colon were not significantly different between pre- and post-treatment individuals in the anthelmintic group.

Table A.3.1: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes (log^2) in the whole gut microbiota between pre- and post-treatment mice in an anthelmintic group.

OTU	Base Mean	Log^2 fold change	Log^2 fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO437	3.88	-3.99	1.25	-3.18	0.001452	0.020668	*Firmicutes*	*Clostridia*
DENOVO425	1.91	-3.78	1.19	-3.18	0.001455	0.020668	*Firmicutes*	*Clostridia*
DENOVO757	3.76	-2.68	0.79	-3.39	0.000706	0.013732	*Firmicutes*	*Clostridia*
DENOVO750	0.71	-2.48	0.80	-3.09	0.002005	0.026772	*Firmicutes*	*Clostridia*
DENOVO79	2.22	2.23	0.77	2.91	0.003568	0.039829	*Firmicutes*	*Clostridia*
DENOVO129	12.67	-2.81	0.88	-3.18	0.001457	0.020668	*Firmicutes*	*Clostridia*
DENOVO188	6.00	-3.47	0.91	-3.81	0.000141	0.003832	*Firmicutes*	*Clostridia*
DENOVO158	39.65	-8.00	1.23	-6.49	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO391	4.15	-3.67	0.80	-4.56	0.000005	0.000262	*Firmicutes*	*Clostridia*
DENOVO286	30.72	-4.21	1.04	-4.04	0.000053	0.001690	*Firmicutes*	*Clostridia*
DENOVO341	6.75	4.45	0.95	4.70	0.000003	0.000162	*Firmicutes*	*Clostridia*
DENOVO339	3.36	-3.11	0.96	-3.24	0.001178	0.019106	*Firmicutes*	*Clostridia*
DENOVO212	16.77	-3.18	0.77	-4.15	0.000033	0.001489	*Firmicutes*	*Clostridia*
DENOVO544	5.80	-2.59	0.74	-3.48	0.000501	0.010335	*Firmicutes*	*Clostridia*
DENOVO415	10.10	-4.49	1.10	-4.08	0.000045	0.001662	*Firmicutes*	*Clostridia*
DENOVO523	1.80	-2.21	0.72	-3.08	0.002060	0.026828	*Firmicutes*	*Clostridia*
DENOVO204	10.81	-1.95	0.64	-3.04	0.002382	0.029497	*Firmicutes*	*Clostridia*
DENOVO103	65.90	-8.25	1.16	-7.10	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO306	9.65	-3.62	1.06	-3.42	0.000629	0.012605	*Firmicutes*	*Clostridia*
DENOVO996	3.79	-2.27	0.58	-3.94	0.000081	0.002290	*Firmicutes*	*Clostridia*
DENOVO575	1.26	-2.36	0.66	-3.59	0.000328	0.007445	*Firmicutes*	*Clostridia*
DENOVO233	7.68	-2.96	0.73	-4.09	0.000044	0.001662	*Firmicutes*	*Clostridia*
DENOVO656	3.52	-4.47	0.90	-4.94	0.000001	0.000067	*Firmicutes*	*Clostridia*

DENOVO385	5.31	-2.71	0.82	-3.30	0.000959	0.016742	*Firmicutes*	*Clostridia*
DENOVO402	0.81	-3.25	1.06	-3.07	0.002127	0.026828	*Firmicutes*	*Clostridia*
DENOVO805	1.70	-3.15	1.02	-3.08	0.002098	0.026828	*Firmicutes*	*Clostridia*
DENOVO181	26.88	-4.23	1.14	-3.73	0.000191	0.004830	*Bacteroidetes*	*Bacteroidia*
DENOVO35	22.04	-3.13	1.10	-2.83	0.004652	0.047288	*Bacteroidetes*	*Bacteroidia*
DENOVO88	4.24	-3.12	1.06	-2.95	0.003214	0.036481	*Bacteroidetes*	*Bacteroidia*
DENOVO2673	2.02	4.01	0.98	4.08	0.000046	0.001662	*Bacteroidetes*	*Bacteroidia*
DENOVO228	6.43	2.01	0.67	3.02	0.002486	0.029705	*Bacteroidetes*	*Bacteroidia*
DENOVO91	26.67	3.39	0.90	3.76	0.000169	0.004436	*Bacteroidetes*	*Bacteroidia*
DENOVO992	2.21	2.79	0.85	3.30	0.000959	0.016742	*Bacteroidetes*	*Bacteroidia*
DENOVO1027	3.78	2.90	0.79	3.68	0.000237	0.005749	*Bacteroidetes*	*Bacteroidia*
DENOVO12	71.19	4.46	0.77	5.81	0.000000	0.000001	*Bacteroidetes*	*Bacteroidia*
DENOVO38	54.67	2.43	0.61	4.01	0.000062	0.001826	*Bacteroidetes*	*Bacteroidia*
DENOVO821	2.70	2.83	0.86	3.27	0.001061	0.017627	*Bacteroidetes*	*Bacteroidia*
DENOVO122	17.80	2.47	0.85	2.90	0.003734	0.041014	*Bacteroidetes*	*Bacteroidia*
DENOVO72	40.63	2.00	0.55	3.67	0.000245	0.005749	*Bacteroidetes*	*Bacteroidia*
DENOVO1362	1.75	2.56	0.77	3.34	0.000827	0.015650	*Bacteroidetes*	*Bacteroidia*
DENOVO45	117.40	1.70	0.54	3.13	0.001732	0.024072	*Bacteroidetes*	*Bacteroidia*
DENOVO120	24.41	2.77	0.63	4.36	0.000013	0.000635	*Bacteroidetes*	*Bacteroidia*
DENOVO57	42.31	3.31	0.54	6.18	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO37	41.94	4.33	0.81	5.34	0.000000	0.000011	*Bacteroidetes*	*Bacteroidia*
DENOVO43	134.06	3.90	0.63	6.17	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO29	55.47	3.91	0.75	5.20	0.000000	0.000019	*Bacteroidetes*	*Bacteroidia*
DENOVO1494	1.23	2.35	0.67	3.53	0.000412	0.009059	*Bacteroidetes*	*Bacteroidia*
DENOVO1484	1.68	2.59	0.92	2.83	0.004645	0.047288	*Bacteroidetes*	*Bacteroidia*
DENOVO501	2.55	3.35	1.01	3.33	0.000875	0.016107	*Bacteroidetes*	*Bacteroidia*
DENOVO118	42.05	3.72	1.16	3.21	0.001314	0.020102	*Firmicutes*	*Clostridia*
DENOVO1154	1.94	-3.05	0.95	-3.21	0.001328	0.020102	*Firmicutes*	*Clostridia*
DENOVO223	15.22	-3.54	0.88	-4.03	0.000055	0.001690	*Firmicutes*	*Clostridia*

DENOVO610	1.27	-2.54	0.85	-2.97	0.002936	0.033884	*Firmicutes*	*Clostridia*
DENOVO668	2.88	-2.05	0.67	-3.03	0.002430	0.029548	*Firmicutes*	*Clostridia*
DENOVO75	15.24	-2.19	0.54	-4.06	0.000050	0.001690	*Firmicutes*	*Clostridia*
DENOVO1022	2.65	-2.41	0.69	-3.50	0.000459	0.009775	*Firmicutes*	*Clostridia*
DENOVO125	41.03	-3.60	0.74	-4.88	0.000001	0.000082	*Firmicutes*	*Clostridia*
DENOVO713	0.95	-2.93	0.97	-3.01	0.002587	0.030380	*Firmicutes*	*Clostridia*
DENOVO102	14.99	3.98	1.38	2.88	0.004019	0.043441	*Firmicutes*	*Erysipelotrichia*
DENOVO16	114.04	-3.63	1.27	-2.87	0.004091	0.043535	*Tenericutes*	*Mollicutes*
DENOVO307	5.27	1.55	0.54	2.86	0.004177	0.043766	*Actinobacteria*	*Actinobacteria*
DENOVO303	2.77	2.99	0.73	4.07	0.000046	0.001662	*Actinobacteria*	*Actinobacteria*
DENOVO255	4.82	4.94	1.03	4.77	0.000002	0.000124	*Actinobacteria*	*Actinobacteria*
DENOVO269	4.35	-1.91	0.62	-3.09	0.001986	0.026772	*Proteobacteria*	*Deltaproteobacteria*
DENOVO312	5.69	1.96	0.61	3.21	0.001313	0.020102	*Proteobacteria*	*Deltaproteobacteria*
DENOVO7	75.20	3.23	0.98	3.28	0.001022	0.017402	*Proteobacteria*	*Gammaproteobacteria*
DENOVO159	20.33	5.95	1.29	4.60	0.000004	0.000235	*Proteobacteria*	*Gammaproteobacteria*

Table A.3.2: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances ($p < 0.05$) and their respective fold changes ($\log^2$) in the whole gut microbiota between pre- and post-treatment mice in a control group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO437	3.88	-3.99	1.25	-3.18	0.001452	0.020668	*Firmicutes*	*Clostridia*
DENOVO425	1.91	-3.78	1.19	-3.18	0.001455	0.020668	*Firmicutes*	*Clostridia*
DENOVO757	3.76	-2.68	0.79	-3.39	0.000706	0.013732	*Firmicutes*	*Clostridia*
DENOVO750	0.71	-2.48	0.80	-3.09	0.002005	0.026772	*Firmicutes*	*Clostridia*
DENOVO79	2.22	2.23	0.77	2.91	0.003568	0.039829	*Firmicutes*	*Clostridia*
DENOVO129	12.67	-2.81	0.88	-3.18	0.001457	0.020668	*Firmicutes*	*Clostridia*

DENOVO188	6.00	-3.47	0.91	-3.81	0.000141	0.003832	*Firmicutes*	*Clostridia*
DENOVO158	39.65	-8.00	1.23	-6.49	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO391	4.15	-3.67	0.80	-4.56	0.000005	0.000262	*Firmicutes*	*Clostridia*
DENOVO286	30.72	-4.21	1.04	-4.04	0.000053	0.001690	*Firmicutes*	*Clostridia*
DENOVO341	6.75	4.45	0.95	4.70	0.000003	0.000162	*Firmicutes*	*Clostridia*
DENOVO339	3.36	-3.11	0.96	-3.24	0.001178	0.019106	*Firmicutes*	*Clostridia*
DENOVO212	16.77	-3.18	0.77	-4.15	0.000033	0.001489	*Firmicutes*	*Clostridia*
DENOVO544	5.80	-2.59	0.74	-3.48	0.000501	0.010335	*Firmicutes*	*Clostridia*
DENOVO415	10.10	-4.49	1.10	-4.08	0.000045	0.001662	*Firmicutes*	*Clostridia*
DENOVO523	1.80	-2.21	0.72	-3.08	0.002060	0.026828	*Firmicutes*	*Clostridia*
DENOVO204	10.81	-1.95	0.64	-3.04	0.002382	0.029497	*Firmicutes*	*Clostridia*
DENOVO103	65.90	-8.25	1.16	-7.10	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO306	9.65	-3.62	1.06	-3.42	0.000629	0.012605	*Firmicutes*	*Clostridia*
DENOVO996	3.79	-2.27	0.58	-3.94	0.000081	0.002290	*Firmicutes*	*Clostridia*
DENOVO575	1.26	-2.36	0.66	-3.59	0.000328	0.007445	*Firmicutes*	*Clostridia*
DENOVO233	7.68	-2.96	0.73	-4.09	0.000044	0.001662	*Firmicutes*	*Clostridia*
DENOVO656	3.52	-4.47	0.90	-4.94	0.000001	0.000067	*Firmicutes*	*Clostridia*
DENOVO385	5.31	-2.71	0.82	-3.30	0.000959	0.016742	*Firmicutes*	*Clostridia*
DENOVO402	0.81	-3.25	1.06	-3.07	0.002127	0.026828	*Firmicutes*	*Clostridia*
DENOVO805	1.70	-3.15	1.02	-3.08	0.002098	0.026828	*Firmicutes*	*Clostridia*
DENOVO181	26.88	-4.23	1.14	-3.73	0.000191	0.004830	*Bacteroidetes*	*Bacteroidia*
DENOVO35	22.04	-3.13	1.10	-2.83	0.004652	0.047288	*Bacteroidetes*	*Bacteroidia*
DENOVO88	4.24	-3.12	1.06	-2.95	0.003214	0.036481	*Bacteroidetes*	*Bacteroidia*
DENOVO2673	2.02	4.01	0.98	4.08	0.000046	0.001662	*Bacteroidetes*	*Bacteroidia*
DENOVO228	6.43	2.01	0.67	3.02	0.002486	0.029705	*Bacteroidetes*	*Bacteroidia*
DENOVO91	26.67	3.39	0.90	3.76	0.000169	0.004436	*Bacteroidetes*	*Bacteroidia*
DENOVO992	2.21	2.79	0.85	3.30	0.000959	0.016742	*Bacteroidetes*	*Bacteroidia*
DENOVO1027	3.78	2.90	0.79	3.68	0.000237	0.005749	*Bacteroidetes*	*Bacteroidia*
DENOVO12	71.19	4.46	0.77	5.81	0.000000	0.000001	*Bacteroidetes*	*Bacteroidia*

DENOVO38	54.67	2.43	0.61	4.01	0.000062	0.001826	*Bacteroidetes*	*Bacteroidia*
DENOVO821	2.70	2.83	0.86	3.27	0.001061	0.017627	*Bacteroidetes*	*Bacteroidia*
DENOVO122	17.80	2.47	0.85	2.90	0.003734	0.041014	*Bacteroidetes*	*Bacteroidia*
DENOVO72	40.63	2.00	0.55	3.67	0.000245	0.005749	*Bacteroidetes*	*Bacteroidia*
DENOVO1362	1.75	2.56	0.77	3.34	0.000827	0.015650	*Bacteroidetes*	*Bacteroidia*
DENOVO45	117.40	1.70	0.54	3.13	0.001732	0.024072	*Bacteroidetes*	*Bacteroidia*
DENOVO120	24.41	2.77	0.63	4.36	0.000013	0.000635	*Bacteroidetes*	*Bacteroidia*
DENOVO57	42.31	3.31	0.54	6.18	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO37	41.94	4.33	0.81	5.34	0.000000	0.000011	*Bacteroidetes*	*Bacteroidia*
DENOVO43	134.06	3.90	0.63	6.17	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO29	55.47	3.91	0.75	5.20	0.000000	0.000019	*Bacteroidetes*	*Bacteroidia*
DENOVO1494	1.23	2.35	0.67	3.53	0.000412	0.009059	*Bacteroidetes*	*Bacteroidia*
DENOVO1484	1.68	2.59	0.92	2.83	0.004645	0.047288	*Bacteroidetes*	*Bacteroidia*
DENOVO501	2.55	3.35	1.01	3.33	0.000875	0.016107	*Bacteroidetes*	*Bacteroidia*
DENOVO118	42.05	3.72	1.16	3.21	0.001314	0.020102	*Firmicutes*	*Clostridia*
DENOVO1154	1.94	-3.05	0.95	-3.21	0.001328	0.020102	*Firmicutes*	*Clostridia*
DENOVO223	15.22	-3.54	0.88	-4.03	0.000055	0.001690	*Firmicutes*	*Clostridia*
DENOVO610	1.27	-2.54	0.85	-2.97	0.002936	0.033884	*Firmicutes*	*Clostridia*
DENOVO668	2.88	-2.05	0.67	-3.03	0.002430	0.029548	*Firmicutes*	*Clostridia*
DENOVO75	15.24	-2.19	0.54	-4.06	0.000050	0.001690	*Firmicutes*	*Clostridia*
DENOVO1022	2.65	-2.41	0.69	-3.50	0.000459	0.009775	*Firmicutes*	*Clostridia*
DENOVO125	41.03	-3.60	0.74	-4.88	0.000001	0.000082	*Firmicutes*	*Clostridia*
DENOVO713	0.95	-2.93	0.97	-3.01	0.002587	0.030380	*Firmicutes*	*Clostridia*
DENOVO102	14.99	3.98	1.38	2.88	0.004019	0.043441	*Firmicutes*	*Erysipelotrichia*
DENOVO16	114.04	-3.63	1.27	-2.87	0.004091	0.043535	*Tenericutes*	*Mollicutes*
DENOVO307	5.27	1.55	0.54	2.86	0.004177	0.043766	*Actinobacteria*	*Actinobacteria*
DENOVO303	2.77	2.99	0.73	4.07	0.000046	0.001662	*Actinobacteria*	*Actinobacteria*
DENOVO255	4.82	4.94	1.03	4.77	0.000002	0.000124	*Actinobacteria*	*Actinobacteria*
DENOVO269	4.35	-1.91	0.62	-3.09	0.001986	0.026772	*Proteobacteria*	*Deltaproteobacteria*

DENOVO312	5.69	1.96	0.61	3.21	0.001313	0.020102	*Proteobacteria*	*Deltaproteobacteria*
DENOVO7	75.20	3.23	0.98	3.28	0.001022	0.017402	*Proteobacteria*	*Gammaproteobacteria*
DENOVO159	20.33	5.95	1.29	4.60	0.000004	0.000235	*Proteobacteria*	*Gammaproteobacteria*

Table A.3.3: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in the small intestine microbiota between pre- and post-treatment mice in a control group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO37	36.33	5.57	1.63	3.41	0.000653	0.041490	*Bacteroidetes*	*Bacteroidia*
DENOVO4	9582.97	-8.62	1.77	-4.86	0.000001	0.000149	*Tenericutes*	*Mollicutes*

Table A.3.4: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in the caecum microbiota between pre- and post-treatment mice in an anthelmintic group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO23	452.78	-5.82	1.30	-4.49	0.000007	0.008915	*Firmicutes*	*Clostridia*
DENOVO4	548.60	-6.46	1.64	-3.95	0.000079	0.049904	*Tenericutes*	*Mollicutes*

Table A.3.5: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in the caecum microbiota between pre- and post-treatment mice in a control group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class

			error					
DENOVO188	114.38	-4.75	1.40	-3.40	0.000679	0.028053	*Firmicutes*	*Clostridia*
DENOVO158	111.36	-6.10	1.50	-4.07	0.000047	0.008614	*Firmicutes*	*Clostridia*
DENOVO178	66.99	-5.02	1.38	-3.64	0.000267	0.021365	*Firmicutes*	*Clostridia*
DENOVO103	118.72	-5.90	1.47	-4.00	0.000063	0.008614	*Firmicutes*	*Clostridia*
DENOVO656	7.83	-4.04	1.15	-3.51	0.000445	0.026243	*Firmicutes*	*Clostridia*
DENOVO902	21.79	-4.71	1.41	-3.33	0.000872	0.031260	*Firmicutes*	*Clostridia*
DENOVO331	44.35	-4.85	1.46	-3.32	0.000908	0.031260	*Firmicutes*	*Clostridia*
DENOVO2673	5.82	4.87	1.28	3.82	0.000136	0.013996	*Bacteroidetes*	*Bacteroidia*
DENOVO36	151.98	2.43	0.77	3.18	0.001471	0.043393	*Bacteroidetes*	*Bacteroidia*
DENOVO12	86.37	4.14	1.15	3.61	0.000310	0.021365	*Bacteroidetes*	*Bacteroidia*
DENOVO57	42.89	3.03	0.74	4.10	0.000041	0.008614	*Bacteroidetes*	*Bacteroidia*
DENOVO37	69.25	3.78	1.19	3.18	0.001454	0.043393	*Bacteroidetes*	*Bacteroidia*
DENOVO43	194.32	3.37	0.98	3.43	0.000607	0.027832	*Bacteroidetes*	*Bacteroidia*
DENOVO29	81.25	3.89	1.13	3.44	0.000587	0.027832	*Bacteroidetes*	*Bacteroidia*

Table A.3.6: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances ($p < 0.05$) and their respective fold changes ($\log^2$) in the colon microbiota between pre- and post-treatment mice in a control group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO158	85.81	-7.07	1.69	-4.17	0.000030	0.004676	*Firmicutes*	*Clostridia*
DENOVO103	202.61	-7.72	1.64	-4.72	0.000002	0.001123	*Firmicutes*	*Clostridia*
DENOVO565	21.11	-4.06	1.20	-3.37	0.000744	0.038779	*Firmicutes*	*Clostridia*
DENOVO331	22.58	-5.79	1.72	-3.37	0.000740	0.038779	*Firmicutes*	*Clostridia*
DENOVO2673	4.43	4.52	1.31	3.46	0.000538	0.038779	*Bacteroidetes*	*Bacteroidia*
DENOVO120	73.95	2.92	0.86	3.41	0.000649	0.038779	*Bacteroidetes*	*Bacteroidia*

DENOVO57	133.45	3.45	0.88	3.93	0.000085	0.009953	*Bacteroidetes*	*Bacteroidia*
DENOVO43	480.97	4.44	1.02	4.33	0.000015	0.003420	*Bacteroidetes*	*Bacteroidia*
DENOVO29	181.87	4.25	1.25	3.41	0.000641	0.038779	*Bacteroidetes*	*Bacteroidia*

Table A.3.7: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in faeces microbiota between pre- and post-treatment mice in an anthelmintic group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	p-value	Adjusted p-value	Phylum	Class
DENOVO333	3.77	-2.41	0.82	-2.95	0.003198	0.037779	*Firmicutes*	*Clostridia*
DENOVO1011	2.21	-3.73	1.12	-3.34	0.000840	0.016242	*Firmicutes*	*Clostridia*
DENOVO757	32.55	-3.94	1.17	-3.36	0.000780	0.016242	*Firmicutes*	*Clostridia*
DENOVO166	20.44	-3.10	0.79	-3.92	0.000090	0.003562	*Firmicutes*	*Clostridia*
DENOVO837	0.87	-3.04	1.05	-2.91	0.003636	0.039702	*Firmicutes*	*Clostridia*
DENOVO79	32.21	-2.81	0.95	-2.96	0.003071	0.036958	*Firmicutes*	*Clostridia*
DENOVO119	29.13	-2.50	0.56	-4.46	0.000008	0.000538	*Firmicutes*	*Clostridia*
DENOVO44	83.01	-2.81	0.68	-4.11	0.000040	0.002158	*Firmicutes*	*Clostridia*
DENOVO387	2.05	-3.78	0.98	-3.85	0.000119	0.004432	*Firmicutes*	*Clostridia*
DENOVO161	11.06	-3.00	0.90	-3.34	0.000846	0.016242	*Firmicutes*	*Clostridia*
DENOVO373	3.82	-2.62	0.79	-3.32	0.000909	0.016991	*Firmicutes*	*Clostridia*
DENOVO339	4.07	-2.73	0.95	-2.88	0.003920	0.040037	*Firmicutes*	*Clostridia*
DENOVO212	5.32	-3.89	0.97	-4.02	0.000057	0.002719	*Firmicutes*	*Clostridia*
DENOVO523	8.35	-2.43	0.86	-2.82	0.004809	0.043776	*Firmicutes*	*Clostridia*
DENOVO84	88.93	-3.26	0.96	-3.38	0.000728	0.016242	*Firmicutes*	*Clostridia*
DENOVO758	1.32	-3.56	1.19	-3.00	0.002691	0.035583	*Firmicutes*	*Clostridia*
DENOVO614	3.98	-4.03	1.24	-3.24	0.001195	0.020700	*Firmicutes*	*Clostridia*
DENOVO180	15.70	-1.90	0.60	-3.19	0.001434	0.023195	*Firmicutes*	*Clostridia*

DENOVO48	129.55	-1.91	0.65	-2.94	0.003299	0.037779	*Firmicutes*	*Clostridia*
DENOVO486	8.41	-2.45	0.75	-3.27	0.001091	0.019358	*Firmicutes*	*Clostridia*
DENOVO788	0.86	-2.53	0.83	-3.04	0.002335	0.032513	*Firmicutes*	*Clostridia*
DENOVO481	2.27	-2.46	0.87	-2.81	0.004913	0.044155	*Firmicutes*	*Clostridia*
DENOVO719	4.79	-2.42	0.78	-3.08	0.002040	0.029560	*Firmicutes*	*Clostridia*
DENOVO548	2.03	-2.25	0.81	-2.78	0.005404	0.046225	*Firmicutes*	*Clostridia*
DENOVO344	5.85	-2.15	0.76	-2.82	0.004742	0.043721	*Firmicutes*	*Clostridia*
DENOVO769	2.37	-2.80	0.84	-3.35	0.000822	0.016242	*Firmicutes*	*Clostridia*
DENOVO304	13.20	-3.42	0.93	-3.70	0.000218	0.007038	*Firmicutes*	*Clostridia*
DENOVO406	62.16	-2.33	0.81	-2.87	0.004167	0.041094	*Firmicutes*	*Clostridia*
DENOVO1152	2.91	-3.23	0.96	-3.37	0.000764	0.016242	*Firmicutes*	*Clostridia*
DENOVO805	2.00	-3.82	1.13	-3.37	0.000758	0.016242	*Firmicutes*	*Clostridia*
DENOVO1142	1.20	-2.90	0.96	-3.01	0.002589	0.035355	*Firmicutes*	*Clostridia*
DENOVO478	1.47	-2.31	0.74	-3.14	0.001687	0.026602	*Firmicutes*	*Clostridia*
DENOVO465	1.40	-2.28	0.76	-2.99	0.002784	0.035583	*Firmicutes*	*Clostridia*
DENOVO256	4.37	3.10	0.85	3.63	0.000278	0.008232	*Bacteroidetes*	*Flavobacteriia*
DENOVO420	4.24	4.81	1.11	4.35	0.000014	0.000808	*Bacteroidetes*	*Flavobacteriia*
DENOVO328	1.95	3.79	1.15	3.29	0.001001	0.018222	*Bacteroidetes*	*Flavobacteriia*
DENOVO351	2.78	3.45	0.96	3.60	0.000324	0.008855	*Bacteroidetes*	*Flavobacteriia*
DENOVO244	11.93	4.50	0.94	4.80	0.000002	0.000127	*Bacteroidetes*	*Sphingobacteriia*
DENOVO584	2.57	3.30	1.16	2.85	0.004391	0.041564	*Bacteroidetes*	*Sphingobacteriia*
DENOVO277	4.63	-1.75	0.62	-2.83	0.004636	0.043308	*Bacteroidetes*	*Bacteroidia*
DENOVO1596	0.87	2.57	0.89	2.88	0.003947	0.040037	*Bacteroidetes*	*Bacteroidia*
DENOVO578	1.55	-2.26	0.78	-2.90	0.003747	0.039702	*Firmicutes*	*Clostridia*
DENOVO294	6.37	-2.34	0.85	-2.76	0.005703	0.047640	*Firmicutes*	*Clostridia*
DENOVO661	5.99	-1.92	0.65	-2.94	0.003298	0.037779	*Firmicutes*	*Clostridia*
DENOVO85	29.77	-1.95	0.64	-3.05	0.002267	0.032197	*Firmicutes*	*Clostridia*
DENOVO811	1.62	-2.81	1.02	-2.77	0.005673	0.047640	*Firmicutes*	*Clostridia*
DENOVO772	2.68	-2.12	0.72	-2.93	0.003422	0.037964	*Firmicutes*	*Clostridia*

DENOVO310	5.14	-2.23	0.75	-2.98	0.002903	0.036161	*Firmicutes*	*Clostridia*
DENOVO668	6.47	-2.74	0.92	-2.99	0.002807	0.035583	*Firmicutes*	*Clostridia*
DENOVO75	24.76	-1.91	0.67	-2.85	0.004309	0.041481	*Firmicutes*	*Clostridia*
DENOVO1022	1.00	-2.81	0.96	-2.93	0.003405	0.037964	*Firmicutes*	*Clostridia*
DENOVO963	2.75	-2.50	0.84	-2.97	0.002987	0.036564	*Firmicutes*	*Clostridia*
DENOVO471	4.25	-2.42	0.64	-3.77	0.000162	0.005481	*Firmicutes*	*Clostridia*
DENOVO19	126.01	-1.67	0.58	-2.87	0.004142	0.041094	*Firmicutes*	*Clostridia*
DENOVO117	4.15	3.33	0.96	3.47	0.000518	0.013126	*Firmicutes*	*Bacilli*
DENOVO25	338.44	-1.61	0.51	-3.13	0.001725	0.026602	*Candidatus Saccharibacteria*	*Saccharibacteria*
DENOVO417	3.49	3.08	1.08	2.85	0.004323	0.041481	*Proteobacteria*	*Betaproteobacteria*
DENOVO587	2.69	4.02	1.26	3.19	0.001437	0.023195	*Proteobacteria*	*Betaproteobacteria*
DENOVO436	5.07	4.07	1.04	3.93	0.000084	0.003517	*Proteobacteria*	*Betaproteobacteria*
DENOVO651	2.47	3.43	1.23	2.79	0.005292	0.045824	*Proteobacteria*	*Betaproteobacteria*
DENOVO452	2.42	2.67	0.95	2.79	0.005230	0.045824	*Proteobacteria*	*Gammaproteobacteria*
DENOVO483	1.89	3.14	0.93	3.37	0.000738	0.016242	*Proteobacteria*	*Gammaproteobacteria*
DENOVO148	10.05	3.36	0.67	5.03	0.000000	0.000043	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1353	4.23	3.35	1.15	2.90	0.003743	0.039702	*Proteobacteria*	*Gammaproteobacteria*
DENOVO430	6.14	4.40	1.16	3.81	0.000139	0.004926	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1	44.18	2.59	0.86	2.99	0.002753	0.035583	*Proteobacteria*	*Gammaproteobacteria*
DENOVO874	33.43	3.23	0.79	4.07	0.000046	0.002345	*Proteobacteria*	*Gammaproteobacteria*
DENOVO159	5.88	4.55	0.83	5.46	0.000000	0.000007	*Proteobacteria*	*Gammaproteobacteria*
DENOVO163	29.88	3.21	0.81	3.98	0.000070	0.003121	*Proteobacteria*	*Gammaproteobacteria*
DENOVO737	2.78	3.84	1.14	3.38	0.000716	0.016242	*Proteobacteria*	*Gammaproteobacteria*
DENOVO427	4.79	3.49	1.13	3.09	0.002008	0.029560	*Proteobacteria*	*Gammaproteobacteria*
DENOVO513	2.34	3.44	1.07	3.21	0.001350	0.022813	*Proteobacteria*	*Gammaproteobacteria*
DENOVO270	12.69	3.56	0.98	3.64	0.000277	0.008232	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1139	5.94	5.25	1.10	4.75	0.000002	0.000143	*Proteobacteria*	*Gammaproteobacteria*
DENOVO560	3.04	3.79	1.36	2.80	0.005155	0.045755	*Proteobacteria*	*Gammaproteobacteria*

DENOVO684	14.76	6.09	1.20	5.08	0.000000	0.000039	*Proteobacteria*	*Gammaproteobacteria*
DENOVO179	31.14	3.50	0.68	5.13	0.000000	0.000035	*Proteobacteria*	*Gammaproteobacteria*
DENOVO151	21.28	6.64	1.02	6.48	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO169	26.46	4.27	0.75	5.70	0.000000	0.000002	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1224	1.31	3.02	0.97	3.13	0.001761	0.026602	*Proteobacteria*	*Gammaproteobacteria*
DENOVO47	109.39	5.85	0.90	6.47	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO2389	3.39	4.37	1.23	3.57	0.000361	0.009500	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1331	1.26	3.19	1.10	2.89	0.003875	0.040037	*Proteobacteria*	*Gammaproteobacteria*
DENOVO6	704.33	5.05	0.79	6.36	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO912	1.28	3.25	0.90	3.61	0.000305	0.008655	*Proteobacteria*	*Alphaproteobacteria*

Table A.3.8: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in faeces microbiota between pre- and post-treatment mice in a control group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	p-value	Adjusted p-value	Phylum	Class
DENOVO188	114.38	-4.75	1.40	-3.40	0.000679	0.028053	*Firmicutes*	*Clostridia*
DENOVO158	111.36	-6.10	1.50	-4.07	0.000047	0.008614	*Firmicutes*	*Clostridia*
DENOVO178	66.99	-5.02	1.38	-3.64	0.000267	0.021365	*Firmicutes*	*Clostridia*
DENOVO103	118.72	-5.90	1.47	-4.00	0.000063	0.008614	*Firmicutes*	*Clostridia*
DENOVO656	7.83	-4.04	1.15	-3.51	0.000445	0.026243	*Firmicutes*	*Clostridia*
DENOVO902	21.79	-4.71	1.41	-3.33	0.000872	0.031260	*Firmicutes*	*Clostridia*
DENOVO331	44.35	-4.85	1.46	-3.32	0.000908	0.031260	*Firmicutes*	*Clostridia*
DENOVO2673	5.82	4.87	1.28	3.82	0.000136	0.013996	*Bacteroidetes*	*Bacteroidia*
DENOVO36	151.98	2.43	0.77	3.18	0.001471	0.043393	*Bacteroidetes*	*Bacteroidia*
DENOVO12	86.37	4.14	1.15	3.61	0.000310	0.021365	*Bacteroidetes*	*Bacteroidia*
DENOVO57	42.89	3.03	0.74	4.10	0.000041	0.008614	*Bacteroidetes*	*Bacteroidia*

DENOVO37	69.25	3.78	1.19	3.18	0.001454	0.043393	*Bacteroidetes*	*Bacteroidia*
DENOVO43	194.32	3.37	0.98	3.43	0.000607	0.027832	*Bacteroidetes*	*Bacteroidia*
DENOVO29	81.25	3.89	1.13	3.44	0.000587	0.027832	*Bacteroidetes*	*Bacteroidia*

Appendix A.4: Supplementary tables of data presented in Chapter 4

Statistical outputs of analyses to test for OTUs that significantly differed in abundance between pre- and post-treatment individuals in an antibiotic and a control group, for microbiota of the whole gut (three gut sections combined), small intestine, caecum, colon and faeces. OTUs were grouped by microbial phylum and class. Briefly, DESeq was used to identify significantly different (p <0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing pre- and post-treatment mice. Below are the tables resulting from these analyses.

Note: Data from the control group can be seen in Appendix A.3

Table A.4.1: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances ($p < 0.05$) and their respective fold changes ($\log^2$) in the whole gut microbiota between pre- and post-treatment mice in an antibiotic group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO680	69.73	-5.52	1.69	-3.26	0.001100	0.036460	*Firmicutes*	*Clostridia*
DENOVO158	74.36	-6.25	2.03	-3.07	0.002123	0.046590	*Firmicutes*	*Clostridia*
DENOVO660	69.50	-5.43	1.67	-3.25	0.001155	0.036460	*Firmicutes*	*Clostridia*
DENOVO339	61.76	-5.22	1.62	-3.23	0.001224	0.036460	*Firmicutes*	*Clostridia*
DENOVO306	158.17	-7.25	1.98	-3.67	0.000245	0.017021	*Firmicutes*	*Clostridia*
DENOVO92	611.76	-5.63	1.61	-3.49	0.000475	0.022383	*Firmicutes*	*Clostridia*
DENOVO511	188.90	-6.22	1.98	-3.14	0.001667	0.042328	*Firmicutes*	*Clostridia*
DENOVO199	1363.14	-6.52	1.67	-3.91	0.000091	0.009488	*Firmicutes*	*Clostridia*
DENOVO1148	364.03	-5.37	1.73	-3.11	0.001876	0.043470	*Firmicutes*	*Clostridia*
DENOVO39	2571.55	-4.98	1.56	-3.20	0.001393	0.038712	*Firmicutes*	*Clostridia*
DENOVO1194	130.21	-7.06	1.97	-3.58	0.000342	0.020349	*Firmicutes*	*Clostridia*
DENOVO35	516.24	-7.23	1.90	-3.81	0.000140	0.011647	*Bacteroidetes*	*Bacteroidia*
DENOVO25	188.65	-4.64	1.48	-3.13	0.001726	0.042328	*Candidatus Saccharibacteria*	*Saccharibacteria*
DENOVO168	148.55	-6.67	1.36	-4.89	0.000001	0.000142	*Firmicutes*	*Erysipelotrichia*
DENOVO31	1817.94	-9.01	1.58	-5.71	0.000000	0.000002	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO484	28.08	-5.70	1.71	-3.34	0.000848	0.035345	*Actinobacteria*	*Actinobacteria*
DENOVO1	1996.17	9.09	1.48	6.14	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO97	53.00	5.34	1.63	3.28	0.001045	0.036460	*Proteobacteria*	*Gammaproteobacteria*
DENOVO429	545.48	6.59	1.89	3.49	0.000483	0.022383	*Proteobacteria*	*Gammaproteobacteria*

Table A.4.2: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in the small intestine microbiota between pre- and post-treatment mice in an antibiotic group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO17	360.48	-8.05	2.08	-3.87	0.000108	0.032862	*Firmicutes*	*Bacilli*
DENOVO16	429.53	-8.03	1.95	-4.12	0.000038	0.023286	*Tenericutes*	*Mollicutes*

Table A.4.3: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in the caecum microbiota between pre- and post-treatment mice in an antibiotic group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO33	567.15	-8.31	2.02	-4.12	0.000038	0.017436	*Firmicutes*	*Clostridia*
DENOVO13	28.94	6.93	1.80	3.86	0.000116	0.028157	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO31	446.97	-8.57	1.70	-5.05	0.000000	0.000547	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO7	7945.59	7.57	1.85	4.09	0.000043	0.017436	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1	18050.34	7.28	1.88	3.87	0.000111	0.028157	*Proteobacteria*	*Gammaproteobacteria*

Table A.4.4: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in the colon microbiota between pre- and post-treatment mice in an antibiotic group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class

DENOVO757	30.07	-5.87	1.76	-3.33	0.000865	0.029700	*Firmicutes*	*Clostridia*
DENOVO79	138.35	-6.82	1.65	-4.13	0.000036	0.007461	*Firmicutes*	*Clostridia*
DENOVO146	67.01	-5.70	1.86	-3.06	0.002221	0.038128	*Firmicutes*	*Clostridia*
DENOVO979	31.44	-5.91	1.75	-3.38	0.000731	0.029700	*Firmicutes*	*Clostridia*
DENOVO158	62.88	-6.05	1.99	-3.03	0.002429	0.038485	*Firmicutes*	*Clostridia*
DENOVO1717	47.86	-5.44	1.89	-2.88	0.004020	0.048708	*Firmicutes*	*Clostridia*
DENOVO318	160.33	-7.50	2.10	-3.57	0.000356	0.022092	*Firmicutes*	*Clostridia*
DENOVO92	132.86	-4.82	1.55	-3.11	0.001858	0.034800	*Firmicutes*	*Clostridia*
DENOVO280	41.90	-5.69	1.92	-2.96	0.003029	0.041602	*Firmicutes*	*Clostridia*
DENOVO199	121.08	-5.32	1.63	-3.27	0.001087	0.031995	*Firmicutes*	*Clostridia*
DENOVO445	101.21	-6.21	1.97	-3.16	0.001602	0.032997	*Firmicutes*	*Clostridia*
DENOVO216	180.07	-7.82	2.07	-3.77	0.000164	0.016901	*Firmicutes*	*Clostridia*
DENOVO17	310.47	-6.43	2.02	-3.19	0.001441	0.032978	*Firmicutes*	*Bacilli*
DENOVO117	21.47	5.47	1.89	2.90	0.003742	0.048176	*Firmicutes*	*Bacilli*
DENOVO168	40.79	-5.08	1.70	-2.98	0.002846	0.041602	*Firmicutes*	*Erysipelotrichia*
DENOVO13	59.98	6.71	1.91	3.52	0.000429	0.022092	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO1	2398.16	6.41	1.99	3.22	0.001271	0.032737	*Proteobacteria*	*Gammaproteobacteria*

Table A.4.5: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) in faeces microbiota between pre- and post-treatment mice in an antibiotic group.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO537	3.62	-3.43	0.84	-4.11	0.000039	0.001052	*Firmicutes*	*Clostridia*
DENOVO245	6.94	-2.58	0.93	-2.79	0.005338	0.040462	*Firmicutes*	*Clostridia*
DENOVO162	23.21	-2.04	0.70	-2.93	0.003436	0.028848	*Firmicutes*	*Clostridia*
DENOVO757	45.19	-5.09	1.12	-4.53	0.000006	0.000234	*Firmicutes*	*Clostridia*

DENOVO975	2.22	-3.25	1.08	-3.01	0.002592	0.023569	*Firmicutes*	*Clostridia*
DENOVO663	2.29	-3.72	0.96	-3.86	0.000111	0.002416	*Firmicutes*	*Clostridia*
DENOVO630	4.80	-4.70	0.85	-5.53	0.000000	0.000004	*Firmicutes*	*Clostridia*
DENOVO387	5.65	-4.70	1.10	-4.27	0.000020	0.000603	*Firmicutes*	*Clostridia*
DENOVO158	24.24	-5.88	1.86	-3.16	0.001580	0.016880	*Firmicutes*	*Clostridia*
DENOVO391	1.45	-3.18	0.95	-3.36	0.000787	0.010383	*Firmicutes*	*Clostridia*
DENOVO109	15.93	-3.83	1.02	-3.75	0.000177	0.003226	*Firmicutes*	*Clostridia*
DENOVO321	6.51	-2.91	0.79	-3.69	0.000221	0.003820	*Firmicutes*	*Clostridia*
DENOVO161	21.93	3.18	0.92	3.46	0.000532	0.007620	*Firmicutes*	*Clostridia*
DENOVO212	11.79	-3.94	0.84	-4.71	0.000003	0.000121	*Firmicutes*	*Clostridia*
DENOVO170	13.97	2.50	0.91	2.73	0.006294	0.045545	*Firmicutes*	*Clostridia*
DENOVO544	2.84	-3.43	0.91	-3.78	0.000159	0.003047	*Firmicutes*	*Clostridia*
DENOVO1030	4.01	-2.81	0.80	-3.51	0.000452	0.006755	*Firmicutes*	*Clostridia*
DENOVO564	2.07	-2.79	0.96	-2.90	0.003701	0.030009	*Firmicutes*	*Clostridia*
DENOVO862	1.18	-3.76	1.36	-2.76	0.005721	0.042312	*Firmicutes*	*Clostridia*
DENOVO204	23.13	-2.78	0.93	-2.98	0.002907	0.025744	*Firmicutes*	*Clostridia*
DENOVO103	109.77	-5.74	1.20	-4.77	0.000002	0.000094	*Firmicutes*	*Clostridia*
DENOVO48	35.31	-1.93	0.52	-3.67	0.000239	0.004015	*Firmicutes*	*Clostridia*
DENOVO219	5.54	-2.75	0.91	-3.04	0.002377	0.022532	*Firmicutes*	*Clostridia*
DENOVO486	2.81	-2.64	0.77	-3.42	0.000627	0.008611	*Firmicutes*	*Clostridia*
DENOVO288	11.42	-2.55	0.78	-3.26	0.001131	0.013127	*Firmicutes*	*Clostridia*
DENOVO996	4.62	-3.74	0.85	-4.39	0.000011	0.000362	*Firmicutes*	*Clostridia*
DENOVO579	11.29	-4.24	0.87	-4.87	0.000001	0.000063	*Firmicutes*	*Clostridia*
DENOVO404	24.34	-3.65	0.73	-4.97	0.000001	0.000041	*Firmicutes*	*Clostridia*
DENOVO413	3.89	-2.79	0.92	-3.04	0.002376	0.022532	*Firmicutes*	*Clostridia*
DENOVO92	25.73	-3.62	1.03	-3.51	0.000451	0.006755	*Firmicutes*	*Clostridia*
DENOVO23	9.51	-2.54	0.88	-2.88	0.003965	0.031391	*Firmicutes*	*Clostridia*
DENOVO304	8.71	-2.65	0.85	-3.13	0.001748	0.017598	*Firmicutes*	*Clostridia*

DENOVO406	42.18	-2.37	0.85	-2.78	0.005411	0.040462	*Firmicutes*	*Clostridia*
DENOVO346	9.85	-3.29	0.87	-3.79	0.000153	0.003032	*Firmicutes*	*Clostridia*
DENOVO104	18.67	-3.14	1.02	-3.06	0.002197	0.021743	*Firmicutes*	*Clostridia*
DENOVO395	3.79	-3.21	0.85	-3.77	0.000163	0.003047	*Firmicutes*	*Clostridia*
DENOVO1152	3.59	-4.34	1.20	-3.62	0.000300	0.004761	*Firmicutes*	*Clostridia*
DENOVO69	61.94	-4.91	0.91	-5.38	0.000000	0.000008	*Firmicutes*	*Clostridia*
DENOVO671	1.77	-3.10	1.14	-2.72	0.006597	0.046938	*Firmicutes*	*Clostridia*
DENOVO14	71.13	-2.30	0.71	-3.27	0.001091	0.012881	*Firmicutes*	*Clostridia*
DENOVO282	67.37	-3.02	0.90	-3.36	0.000772	0.010383	*Firmicutes*	*Clostridia*
DENOVO65	36.76	-2.76	0.69	-4.02	0.000058	0.001402	*Firmicutes*	*Clostridia*
DENOVO687	18.85	-5.40	0.79	-6.79	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO127	41.76	-4.41	0.72	-6.13	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO49	85.91	-4.50	1.10	-4.10	0.000042	0.001077	*Firmicutes*	*Clostridia*
DENOVO465	1.41	-3.12	1.14	-2.74	0.006163	0.045087	*Firmicutes*	*Clostridia*
DENOVO54	16.86	-4.85	1.46	-3.32	0.000895	0.011367	*Bacteroidetes*	*Bacteroidia*
DENOVO128	5.07	-4.91	1.56	-3.14	0.001661	0.017397	*Bacteroidetes*	*Bacteroidia*
DENOVO242	11.50	-5.14	1.17	-4.39	0.000011	0.000362	*Bacteroidetes*	*Bacteroidia*
DENOVO839	7.58	-3.45	1.20	-2.88	0.003944	0.031391	*Bacteroidetes*	*Bacteroidia*
DENOVO68	22.32	-3.78	1.15	-3.29	0.000997	0.012084	*Bacteroidetes*	*Bacteroidia*
DENOVO35	90.23	-8.03	1.57	-5.10	0.000000	0.000026	*Bacteroidetes*	*Bacteroidia*
DENOVO88	14.36	-6.51	1.55	-4.20	0.000027	0.000795	*Bacteroidetes*	*Bacteroidia*
DENOVO1326	1.18	-3.00	1.11	-2.71	0.006696	0.046938	*Bacteroidetes*	*Bacteroidia*
DENOVO2100	2.26	-3.46	1.08	-3.20	0.001398	0.015423	*Bacteroidetes*	*Bacteroidia*
DENOVO2155	1.29	-3.52	1.18	-2.99	0.002807	0.025190	*Bacteroidetes*	*Bacteroidia*
DENOVO91	19.13	-4.03	1.21	-3.32	0.000891	0.011367	*Bacteroidetes*	*Bacteroidia*
DENOVO36	86.09	-3.94	0.89	-4.43	0.000010	0.000339	*Bacteroidetes*	*Bacteroidia*
DENOVO739	14.87	-3.91	0.94	-4.14	0.000035	0.000971	*Bacteroidetes*	*Bacteroidia*
DENOVO343	6.90	-4.40	1.19	-3.71	0.000208	0.003676	*Bacteroidetes*	*Bacteroidia*

DENOVO615	1.45	-3.10	1.02	-3.04	0.002367	0.022532	*Bacteroidetes*	*Bacteroidia*
DENOVO2430	2.57	-3.87	1.07	-3.61	0.000304	0.004761	*Bacteroidetes*	*Bacteroidia*
DENOVO2250	2.52	-2.92	1.08	-2.70	0.006910	0.047945	*Bacteroidetes*	*Bacteroidia*
DENOVO98	72.66	-3.24	1.07	-3.01	0.002582	0.023569	*Bacteroidetes*	*Bacteroidia*
DENOVO93	81.07	-2.20	0.77	-2.85	0.004420	0.034590	*Firmicutes*	*Clostridia*
DENOVO105	22.74	-4.22	1.16	-3.63	0.000285	0.004685	*Firmicutes*	*Clostridia*
DENOVO578	1.99	-2.76	0.80	-3.44	0.000591	0.008289	*Firmicutes*	*Clostridia*
DENOVO287	4.28	4.25	1.31	3.24	0.001194	0.013623	*Firmicutes*	*Clostridia*
DENOVO661	4.27	-3.50	0.78	-4.48	0.000007	0.000275	*Firmicutes*	*Clostridia*
DENOVO131	65.46	4.21	1.06	3.95	0.000077	0.001758	*Firmicutes*	*Clostridia*
DENOVO490	1.31	-2.23	0.79	-2.82	0.004834	0.037394	*Firmicutes*	*Clostridia*
DENOVO626	9.81	-1.99	0.62	-3.23	0.001232	0.013815	*Firmicutes*	*Clostridia*
DENOVO668	3.49	-1.56	0.54	-2.91	0.003665	0.030009	*Firmicutes*	*Clostridia*
DENOVO504	1.40	2.83	0.95	2.97	0.003002	0.025898	*Firmicutes*	*Clostridia*
DENOVO963	2.71	-2.43	0.78	-3.14	0.001680	0.017397	*Firmicutes*	*Clostridia*
DENOVO713	2.08	-3.14	0.90	-3.49	0.000485	0.007093	*Firmicutes*	*Clostridia*
DENOVO17	28.62	-3.53	1.20	-2.94	0.003231	0.027525	*Firmicutes*	*Bacilli*
DENOVO25	178.04	-5.57	1.04	-5.36	0.000000	0.000008	*Candidatus Saccharibacteria*	*Saccharibacteria*
DENOVO585	6.16	4.02	1.05	3.83	0.000129	0.002631	*Firmicutes*	*Erysipelotrichia*
DENOVO330	3.87	-5.00	1.30	-3.84	0.000124	0.002599	*Elusimicrobia*	*Elusimicrobia*
DENOVO31	21.18	-6.65	1.17	-5.67	0.000000	0.000002	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO298	3.06	-5.08	1.25	-4.07	0.000048	0.001190	*Proteobacteria*	*Deltaproteobacteria*
DENOVO177	1.14	-2.72	1.00	-2.71	0.006695	0.046938	*Actinobacteria*	*Actinobacteria*
DENOVO295	3.39	-2.40	0.79	-3.03	0.002411	0.022538	*Actinobacteria*	*Actinobacteria*
DENOVO388	0.91	-2.99	1.02	-2.92	0.003472	0.028848	*Actinobacteria*	*Actinobacteria*
DENOVO206	4.28	-5.63	1.13	-4.97	0.000001	0.000041	*Proteobacteria*	*Deltaproteobacteria*
DENOVO239	6.92	-3.60	1.09	-3.29	0.001005	0.012084	*Proteobacteria*	*Deltaproteobacteria*
DENOVO269	8.96	-6.81	0.95	-7.15	0.000000	0.000000	*Proteobacteria*	*Deltaproteobacteria*

DENOVO349	4.20	-5.64	1.10	-5.12	0.000000	0.000025	*Proteobacteria*	*Deltaproteobacteria*
DENOVO603	2.23	-2.88	0.97	-2.97	0.002956	0.025833	*Proteobacteria*	*Betaproteobacteria*
DENOVO485	1.77	-2.75	0.84	-3.29	0.001005	0.012084	*Proteobacteria*	*Betaproteobacteria*
DENOVO836	4.65	4.02	1.45	2.78	0.005375	0.040462	*Proteobacteria*	*Gammaproteobacteria*
DENOVO7	181.10	-3.35	1.05	-3.19	0.001426	0.015474	*Proteobacteria*	*Gammaproteobacteria*
DENOVO429	14.37	5.70	1.22	4.66	0.000003	0.000140	*Proteobacteria*	*Gammaproteobacteria*
DENOVO151	9.56	4.83	1.22	3.95	0.000078	0.001758	*Proteobacteria*	*Gammaproteobacteria*
DENOVO6	637.37	4.24	0.93	4.56	0.000005	0.000213	*Proteobacteria*	*Gammaproteobacteria*
DENOVO689	2.23	-3.28	1.05	-3.13	0.001752	0.017598	*Proteobacteria*	*Alphaproteobacteria*

Appendix A.5: Supplementary tables of data presented in Chapter 5

Table A.5.1: Sampling regime of wild mice (*Apodemus flavicollis*) gut sections sequenced for comparison of the microbial community with helminth-associated microbiota. The distal colon of Mouse 11 was sequences but was discarded from analyses as it did not meet the criteria for quality filtering (indicated in grey).

Mouse no.	Sex	Breeding status	Stomach	Small intestine	Caecum	Proximal colon	Distal colon
1	Female	Sub-adult	1	1	1	1	1
2	Female	Sub-adult	1	1	1	1	1
3	Female	Sub-adult	1	1	1	1	1
4	Male	Sub-adult	1	1	1	1	1
5	Male	Adult	1	1	1	1	1
6	Male	Adult	1	1	1	1	1
7	Female	Adult	1	1	1	1	1
8	Female	Adult	1	1	1	1	1
9	Female	Adult	1	1	1	1	1
10	Female	Adult	1	1	1	1	1
11	Female	Adult	1	1	1	1	1
12	Male	Adult	1	1	1	1	1
13	Male	Adult	1	1	1	1	1
14	Male	Adult	1	1	1	1	1
15	Female	Sub-adult	1	1	1	1	1
16	Female	Sub-adult	1	1	1	1	1
17	Female	Adult	1	1	1	1	1
18	Male	Sub-adult	1	1	1	1	1
19	Male	Adult	0	1	0	0	0
20	Female	Adult	1	1	0	0	0
21	Male	Adult	1	1	1	0	0
22	Female	Sub-adult	0	1	0	0	0
23	Male	Adult	0	1	0	0	0
24	Male	Adult	1	1	0	0	0

25	Male	Adult	0	1	0	0	0
26	Male	Adult	0	1	1	0	0
27	Male	Adult	1	1	0	0	0
28	Male	Adult	1	1	0	0	0
29	Male	Adult	1	1	0	0	0
30	Male	Adult	1	1	1	0	0
31	Female	Adult	1	1	0	0	0
32	Male	Adult	0	1	1	0	0
TOTAL			**26**	**32**	**22**	**18**	**18**

Table A.5.2: Information regarding the number of individual helminths isolated from 32 *Apodemus flavicollis*, which were pooled into samples, and sequenced for microbiota analyses. Sequences from two samples of *S. frederici* (each of one helminth each), one from Mouse 2 and one from Mouse 8, were discarded from analyses as they did not meet the criteria for quality filtering.

Mouse no.	*A. murissylvatici*		*H. polygyrus*		*H. diminuta*		*M. muris*		*S. frederici*		*T. muris*	
	Samples	**Individuals**	**Samples**	**Individuals**	**Samples**	**Individuals**	**Samples**	**Individuals**	**Samples**	**Individuals**	**Samples**	**Individuals**
1	0	0	0	0	1	35	0	0	0	0	0	0
2	0	0	1	5	1	17	0	0	2	7	0	0
3	0	0	0	0	1	51	0	0	1	8	0	0
4	0	0	1	11	7	97	0	0	2	53	0	0
5	0	0	1	12	1	52	0	0	0	0	1	1
6	0	0	0	0	1	35	0	0	3	53	0	0
7	0	0	0	0	1	2	21	21	0	0	0	0
8	1	1	1	26	2	4	0	0	1	1	1	1
9	0	0	1	18	1	30	0	0	1	4	1	1
10	1	3	0	0	1	25	12	12	0	0	0	0
11	0	0	0	0	1	22	0	0	1	23	1	3
12	0	0	1	22	2	29	0	0	1	6	0	0
13	1	6	1	19	1	38	0	0	2	166	0	0
14	0	0	0	0	2	89	1	1	0	0	1	1
15	1	5	2	57	1	35	0	0	0	0	1	3
16	0	0	1	19	1	69	1	1	0	0	0	0
17	0	0	1	16	1	85	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	2	4	0	0	0	0	0	0
20	0	0	0	0	1	131	0	0	0	0	0	0

21	0	0	0	0	1	85	0	0	1	98	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	1	6	1	31	1	1	0	0	0	0
24	1	1	0	0	1	43	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	1	1	5	27	0	0	0	0	1	5	1	1
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	1	41	7	7	0	0	0	0	0	0
29	4	5	0	0	10	58	0	0	0	0	0	0
30	1	2	0	0	11	63	0	0	7	43	0	0
31	0	0	0	0	1	105	0	0	0	0	0	0
32	0	0	1	12	2	2	0	0	1	20	0	0
TOTAL	**11**	**24**	**19**	**291**	**63**	**1,244**	**36**	**36**	**24**	**487**	**7**	**11**

Table A.5.3: Detailed breakdown of the number of helminths in each sample for each helminth species that was sequenced. Spearman's rank correlation coefficients were calculated to test for significant correlations between number of helminth individuals in a sample and alpha diversity. Alpha diversity was significantly (positively) correlated with number of individual helminths per sample only for *Trichuris muris*.

Sample no.	Mouse no.	Sample	Gut section	No. of worms	Inverse Simpson index	Correlation between no. of worms and inverse Simpson index
1	10	*A. murissylvatici*	Stomach	3	3.19	d.f.= 9, S = 170, p =0.5
2	13	*A. murissylvatici*	Stomach	6	3.95	
3	15	*A. murissylvatici*	Stomach	5	4.48	
4	24	*A. murissylvatici*	Stomach	1	14.58	
5	26	*A. murissylvatici*	Caecum	1	19.09	
6	29	*A. murissylvatici*	Small intestine	2	1.01	
7	29	*A. murissylvatici*	Stomach	1	1.18	
8	29	*A. murissylvatici*	Stomach	1	1	
9	29	*A. murissylvatici*	Stomach	1	1.08	
10	30	*A. murissylvatici*	Stomach	2	3	
11	8	*A. murissylvatici*	Stomach	1	1.81	
12	12	*H. polygyrus*	Small intestine	22	1.66	d.f. = 17, S = 1,100, p = 0.9
13	13	*H. polygyrus*	Small intestine	19	5.27	
14	15	*H. polygyrus*	Small intestine	27	2.26	
15	15	*H. polygyrus*	Small intestine	30	1.99	
16	16	*H. polygyrus*	Small intestine	19	2.52	
17	17	*H. polygyrus*	Small intestine	16	18.52	
18	2	*H. polygyrus*	Small intestine	5	5.3	
19	23	*H. polygyrus*	Small intestine	6	1.33	
20	26	*H. polygyrus*	Small intestine	1	3.78	
21	26	*H. polygyrus*	Small	1	1.18	

			intestine			
22	26	*H. polygyrus*	Small intestine	1	1.15	
23	26	*H. polygyrus*	Small intestine	1	1.19	
24	26	*H. polygyrus*	Small intestine	23	1.75	
25	28	*H. polygyrus*	Small intestine	41	1.02	
26	32	*H. polygyrus*	Small intestine	12	6.92	
27	4	*H. polygyrus*	Small intestine	11	1.69	
28	5	*H. polygyrus*	Small intestine	12	38.72	
29	8	*H. polygyrus*	Small intestine	26	6.8	
30	9	*H. polygyrus*	Small intestine	18	2.44	
31	1	*H. diminuta*	Small intestine	35	2.12	d.f. = 61, S = 38,000, p = 0.5
32	10	*H. diminuta*	Small intestine	25	1	
33	11	*H. diminuta*	Small intestine	22	1.01	
34	12	*H. diminuta*	Small intestine	18	1	
35	12	*H. diminuta*	Small intestine	11	1	
36	13	*H. diminuta*	Small intestine	38	1.01	
37	14	*H. diminuta*	Caecum	4	7.72	
38	14	*H. diminuta*	Small intestine	85	3.2	
39	15	*H. diminuta*	Small intestine	35	1.12	
40	16	*H. diminuta*	Small intestine	69	1.04	
41	17	*H. diminuta*	Small intestine	85	1.04	

42	19	*H. diminuta*	Small intestine	2	1.1	
43	19	*H. diminuta*	Small intestine	2	1.49	
44	2	*H. diminuta*	Small intestine	17	1.01	
45	20	*H. diminuta*	Small intestine	131	1.01	
46	21	*H. diminuta*	Small intestine	85	1	
47	23	*H. diminuta*	Small intestine	31	1.14	
48	24	*H. diminuta*	Small intestine	43	1	
49	28	*H. diminuta*	Small intestine	1	1.08	
50	28	*H. diminuta*	Small intestine	1	1.03	
51	28	*H. diminuta*	Small intestine	1	1.03	
52	28	*H. diminuta*	Small intestine	1	1.04	
53	28	*H. diminuta*	Small intestine	1	1.02	
54	28	*H. diminuta*	Small intestine	1	1.04	
55	28	*H. diminuta*	Small intestine	1	1.03	
56	29	*H. diminuta*	Small intestine	1	1.01	
57	29	*H. diminuta*	Small intestine	1	1.01	
58	29	*H. diminuta*	Small intestine	1	1.02	
59	29	*H. diminuta*	Small intestine	1	1.01	
60	29	*H. diminuta*	Small intestine	1	1.01	
61	29	*H. diminuta*	Small intestine	1	1.05	

62	29	*H. diminuta*	Small intestine	1	1	
63	29	*H. diminuta*	Small intestine	1	1.07	
64	29	*H. diminuta*	Small intestine	1	1.01	
65	29	*H. diminuta*	Small intestine	49	1.01	
66	3	*H. diminuta*	Small intestine	51	13.07	
67	30	*H. diminuta*	Small intestine	1	1.03	
68	30	*H. diminuta*	Small intestine	1	1.15	
69	30	*H. diminuta*	Small intestine	1	1.33	
70	30	*H. diminuta*	Small intestine	1	1.01	
71	30	*H. diminuta*	Small intestine	1	1.12	
72	30	*H. diminuta*	Small intestine	1	1.07	
73	30	*H. diminuta*	Small intestine	1	1	
74	30	*H. diminuta*	Small intestine	1	1.41	
75	30	*H. diminuta*	Small intestine	1	1.1	
76	30	*H. diminuta*	Small intestine	52	3.96	
77	30	*H. diminuta*	Small intestine	2	1.06	
78	31	*H. diminuta*	Small intestine	105	1.01	
79	32	*H. diminuta*	Small intestine	1	11.27	
80	32	*H. diminuta*	Small intestine	1	10.06	
81	4	*H. diminuta*	Small intestine	1	1.07	

82	4	*H. diminuta*	Small intestine	1	1.49	
83	4	*H. diminuta*	Small intestine	1	1.16	
84	4	*H. diminuta*	Small intestine	1	5.85	
85	4	*H. diminuta*	Small intestine	1	1.64	
86	4	*H. diminuta*	Small intestine	91	1.61	
87	4	*H. diminuta*	Small intestine	1	1.56	
88	5	*H. diminuta*	Small intestine	52	2.02	
89	6	*H. diminuta*	Small intestine	35	1.08	
90	7	*H. diminuta*	Small intestine	2	1.01	
91	8	*H. diminuta*	Caecum	2	1.04	
92	8	*H. diminuta*	Small intestine	2	1.03	
93	9	*H. diminuta*	Small intestine	30	1.48	
94	10	*M. muris*	Stomach	1	3.58	NA (all samples $n = 1$)
95	10	*M. muris*	Stomach	1	3.54	
96	10	*M. muris*	Stomach	1	4.64	
97	10	*M. muris*	Stomach	1	4.91	
98	10	*M. muris*	Stomach	1	2.68	
99	10	*M. muris*	Stomach	1	2.58	
100	10	*M. muris*	Stomach	1	3.47	
101	10	*M. muris*	Stomach	1	3.09	
102	10	*M. muris*	Stomach	1	2.66	
103	10	*M. muris*	Stomach	1	4.51	
104	10	*M. muris*	Stomach	1	3.81	
105	10	*M. muris*	Distal colon	1	1.04	
106	14	*M. muris*	Small intestine	1	6.49	

107	16	*M. muris*	Small intestine	1	1.78	
108	23	*M. muris*	Small intestine	1	8.19	
109	7	*M. muris*	Stomach	1	1.15	
110	7	*M. muris*	Stomach	1	1.57	
111	7	*M. muris*	Stomach	1	1.27	
112	7	*M. muris*	Stomach	1	1.32	
113	7	*M. muris*	Stomach	1	1.05	
114	7	*M. muris*	Stomach	1	1.31	
115	7	*M. muris*	Stomach	1	1.27	
116	7	*M. muris*	Stomach	1	1.07	
117	7	*M. muris*	Stomach	1	1.43	
118	7	*M. muris*	Stomach	1	1.01	
119	7	*M. muris*	Stomach	1	1.14	
120	7	*M. muris*	Stomach	1	1.29	
121	7	*M. muris*	Stomach	1	1.19	
122	7	*M. muris*	Stomach	1	1.11	
123	7	*M. muris*	Stomach	1	1.3	
124	7	*M. muris*	Stomach	1	1.13	
125	7	*M. muris*	Stomach	1	1.1	
126	7	*M. muris*	Stomach	1	1.47	
127	7	*M. muris*	Stomach	1	1.27	
128	7	*M. muris*	Stomach	1	1.11	
129	7	*M. muris*	Stomach	1	1.1	
130	11	*S. frederici*	Caecum	23	3.17	d.f. = 20, S = 1,200, p = 0.1
131	12	*S. frederici*	Caecum	6	2.24	
132	13	*S. frederici*	Caecum	160	48.95	
133	13	*S. frederici*	Proximal colon	6	37.17	
134	2	*S. frederici*	Caecum	6	12.51	
135	21	*S. frederici*	Caecum	98	3.32	
136	26	*S. frederici*	Small intestine	5	1.32	
137	3	*S. frederici*	Caecum	8	1.8	

138	30	*S. frederici*	Caecum	1	1.72	
139	30	*S. frederici*	Caecum	1	1.54	
140	30	*S. frederici*	Caecum	1	1.65	
141	30	*S. frederici*	Caecum	1	2.62	
142	30	*S. frederici*	Caecum	1	2.19	
143	30	*S. frederici*	Caecum	1	1.77	
144	30	*S. frederici*	Caecum	37	3	
145	32	*S. frederici*	Caecum	20	1.66	
146	4	*S. frederici*	Caecum	43	18.27	
147	4	*S. frederici*	Caecum	10	26.65	
148	6	*S. frederici*	Caecum	32	12.9	
149	6	*S. frederici*	Caecum	20	22.77	
150	6	*S. frederici*	Proximal colon	1	1.92	
151	9	*S. frederici*	Small intestine	4	21.97	
152	11	*T. muris*	Caecum	3	47.62	d.f. = 5, $S = 12$, $p = 0.03$
153	14	*T. muris*	Caecum	1	32.09	
154	15	*T. muris*	Caecum	3	48.55	
155	26	*T. muris*	Caecum	1	16.77	
156	5	*T. muris*	Caecum	1	27.44	
157	8	*T. muris*	Caecum	1	29.51	
158	9	*T. muris*	Caecum	1	29.19	

Appendix A.6: Supplementary tables of data presented in Chapter 5

Statistical outputs of analyses to test for OTUs that significantly differed in abundance between a given gut section and each helminth species therein. OTUs were grouped by microbial class. Briefly, DESeq was used to identify significantly changing (p <0.05) OTU abundances and their respective fold changes ($\log^2$) when comparing gut location and helminth species. Below are the tables resulting from these analyses.

Table A.6.1: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the stomach and *Aonchotheca murissylvatici.*

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO64	13.67	-4.72	1.85	-2.55	0.010624	0.036903	*Clostridia*	*Clostridiales*
DENOVO87	14.86	-4.74	1.90	-2.50	0.012487	0.041209	*Clostridia*	*Clostridiales*
DENOVO212	47.32	-5.48	2.11	-2.59	0.009604	0.035022	*Erysipelotrichia*	*Erysipelotrichales*
DENOVO193	132.68	-7.97	1.53	-5.22	0.000000	0.000009	*Bacilli*	*Lactobacillales*
DENOVO60	1053.24	-10.75	1.45	-7.40	0.000000	0.000000	*Bacilli*	*Lactobacillales*
DENOVO18	2328.54	-9.17	2.00	-4.59	0.000004	0.000124	*Bacilli*	*Lactobacillales*
DENOVO1372	68.59	-6.94	1.64	-4.24	0.000023	0.000359	*Bacilli*	*Lactobacillales*
DENOVO5	12719.56	-3.81	1.30	-2.94	0.003242	0.016674	*Bacilli*	*Lactobacillales*
DENOVO2164	36.61	-6.05	1.70	-3.56	0.000369	0.002923	*Bacilli*	*Lactobacillales*
DENOVO2	13234.47	-4.46	1.23	-3.62	0.000289	0.002705	*Bacilli*	*Lactobacillales*
DENOVO372	49.51	-5.78	1.78	-3.25	0.001173	0.007040	*Bacilli*	*Lactobacillales*
DENOVO102	83.63	-7.32	1.57	-4.66	0.000003	0.000106	*Bacilli*	*Bacillales*
DENOVO187	25.41	-5.01	2.08	-2.41	0.015904	0.049202	*Deltaproteobacteria*	*Desulfovibrionales*
DENOVO190	22.38	-5.09	1.97	-2.59	0.009728	0.035022	*Deltaproteobacteria*	*Desulfovibrionales*
DENOVO189	78.71	6.93	1.94	3.58	0.000345	0.002854	*Gammaproteobacteria*	*Xanthomonadales*
DENOVO130	20.95	-5.23	1.84	-2.85	0.004380	0.020646	*Gammaproteobacteria*	*Enterobacteriales*
DENOVO65	651.14	-3.95	1.42	-2.78	0.005434	0.023910	*Gammaproteobacteria*	*Pasteurellales*
DENOVO29	1022.02	-6.96	1.59	-4.38	0.000012	0.000267	*Gammaproteobacteria*	*Enterobacteriales*
DENOVO362	306.96	-6.19	2.06	-3.01	0.002636	0.014499	*Gammaproteobacteria*	*Enterobacteriales*
DENOVO38	500.37	-6.65	1.63	-4.08	0.000044	0.000583	*Gammaproteobacteria*	*Enterobacteriales*
DENOVO105	543.95	-6.09	1.69	-3.61	0.000301	0.002705	*Gammaproteobacteria*	*Enterobacteriales*
DENOVO154	33.21	-5.46	2.00	-2.74	0.006204	0.026704	*Gammaproteobacteria*	*Enterobacteriales*

DENOVO393	51.76	-5.35	2.21	-2.42	0.015703	0.049202	*Gammaproteobacteria*	*Pseudomonadales*
DENOVO71	193.72	-6.93	2.09	-3.32	0.000901	0.006371	*Gammaproteobacteria*	*Pseudomonadales*
DENOVO8	21.48	-4.35	1.80	-2.42	0.015600	0.049202	*Deferribacteres*	*Deferribacterales*
DENOVO10	123.07	-6.69	1.93	-3.46	0.000536	0.003927	*Epsilonproteobacteria*	*Campylobacterales*
DENOVO340	41.15	-6.02	1.83	-3.29	0.001012	0.006463	*Actinobacteria*	*Coriobacteriales*
DENOVO191	165.65	-5.92	1.93	-3.07	0.002136	0.012441	*Actinobacteria*	*Actinomycetales*
DENOVO17	42.24	-5.48	2.11	-2.60	0.009236	0.034504	*Bacteroidia*	*Bacteroidales*
DENOVO53	43.68	-5.23	2.08	-2.51	0.012011	0.040309	*Bacteroidia*	*Bacteroidales*
DENOVO129	46.41	-5.31	2.20	-2.42	0.015678	0.049202	*Bacteroidia*	*Bacteroidales*
DENOVO22	938.40	-8.46	1.97	-4.28	0.000018	0.000331	*Bacteroidia*	*Bacteroidales*
DENOVO219	43.82	-5.46	2.12	-2.57	0.010086	0.035661	*Bacteroidia*	*Bacteroidales*
DENOVO205	67.16	-6.05	2.06	-2.94	0.003284	0.016674	*Bacteroidia*	*Bacteroidales*
DENOVO188	56.26	-5.66	2.14	-2.64	0.008173	0.031732	*Bacteroidia*	*Bacteroidales*
DENOVO164	45.94	-5.71	2.04	-2.80	0.005142	0.023137	*Bacteroidia*	*Bacteroidales*
DENOVO258	91.75	-6.05	2.15	-2.81	0.004888	0.022507	*Bacteroidia*	*Bacteroidales*
DENOVO104	123.13	-6.92	1.97	-3.52	0.000432	0.003288	*Bacteroidia*	*Bacteroidales*
DENOVO66	258.26	-7.49	2.01	-3.72	0.000199	0.002076	*Bacteroidia*	*Bacteroidales*
DENOVO146	16.06	-4.88	1.86	-2.62	0.008792	0.033477	*Bacteroidia*	*Bacteroidales*
DENOVO117	167.73	-6.08	2.25	-2.70	0.006954	0.028898	*Bacteroidia*	*Bacteroidales*
DENOVO150	147.39	-6.77	2.07	-3.27	0.001059	0.006552	*Bacteroidia*	*Bacteroidales*
DENOVO31	20.46	-4.98	1.98	-2.51	0.011976	0.040309	*Bacteroidia*	*Bacteroidales*
DENOVO96	143.50	-5.20	1.75	-2.97	0.002986	0.015978	*Bacteroidia*	*Bacteroidales*
DENOVO165	56.68	-6.03	2.00	-3.01	0.002622	0.014499	*Bacteroidia*	*Bacteroidales*
DENOVO58	190.35	-7.76	1.84	-4.23	0.000024	0.000359	*Bacteroidia*	*Bacteroidales*
DENOVO269	32.89	-5.86	1.78	-3.30	0.000970	0.006430	*Bacteroidia*	*Bacteroidales*
DENOVO279	22.89	-5.18	1.93	-2.68	0.007361	0.029743	*Bacteroidia*	*Bacteroidales*
DENOVO101	130.36	-7.54	1.74	-4.33	0.000015	0.000298	*Bacteroidia*	*Bacteroidales*
DENOVO83	60.67	-5.96	2.06	-2.90	0.003708	0.017906	*Bacteroidia*	*Bacteroidales*

DENOVO47	498.98	-8.43	1.90	-4.44	0.000009	0.000227	*Bacteroidia*	*Bacteroidales*
DENOVO107	142.01	-5.91	2.22	-2.67	0.007693	0.030464	*Bacteroidia*	*Bacteroidales*
DENOVO56	74.11	-6.80	1.79	-3.80	0.000144	0.001583	*Bacteroidia*	*Bacteroidales*
DENOVO114	220.07	-7.31	2.02	-3.62	0.000294	0.002705	*Bacteroidia*	*Bacteroidales*
DENOVO109	136.40	-5.44	1.86	-2.92	0.003459	0.017120	*Bacteroidia*	*Bacteroidales*
DENOVO73	298.47	-7.84	1.96	-4.00	0.000062	0.000773	*Bacteroidia*	*Bacteroidales*
DENOVO46	296.41	-8.16	1.72	-4.75	0.000002	0.000081	*Bacteroidia*	*Bacteroidales*
DENOVO19	206.25	-8.49	1.55	-5.47	0.000000	0.000003	*Bacteroidia*	*Bacteroidales*
DENOVO11	309.76	-6.72	1.73	-3.89	0.000100	0.001167	*Bacteroidia*	*Bacteroidales*
DENOVO45	48.80	-6.34	1.77	-3.58	0.000346	0.002854	*Bacteroidia*	*Bacteroidales*
DENOVO120	40.60	-6.03	1.83	-3.30	0.000974	0.006430	*Bacteroidia*	*Bacteroidales*
DENOVO173	61.06	-4.57	1.70	-2.70	0.007006	0.028898	*Bacteroidia*	*Bacteroidales*
DENOVO61	138.27	-7.49	1.80	-4.16	0.000031	0.000443	*Saccharibacteria*	*Saccharibacteria*
DENOVO172	114.80	-7.94	1.42	-5.59	0.000000	0.000002	*Fusobacteriia*	*Fusobacteriales*

Table A.6.2: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the stomach and *Mastophorus muris*.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO430	14.41	-6.89	2.02	-3.41	0.000651	0.002047	*Firmicutes*	*Clostridia*
DENOVO86	7.06	-5.60	2.02	-2.77	0.005659	0.012370	*Firmicutes*	*Clostridia*
DENOVO28	7.84	-6.04	1.64	-3.69	0.000226	0.000796	*Firmicutes*	*Clostridia*
DENOVO484	23.92	-7.56	2.04	-3.70	0.000214	0.000762	*Firmicutes*	*Clostridia*
DENOVO838	67.64	-8.87	2.08	-4.26	0.000020	0.000097	*Firmicutes*	*Clostridia*
DENOVO533	25.30	-8.04	1.75	-4.60	0.000004	0.000025	*Firmicutes*	*Clostridia*
DENOVO95	6.01	-5.17	1.93	-2.69	0.007209	0.015351	*Firmicutes*	*Clostridia*

DENOVO211	19.61	-7.40	2.18	-3.40	0.000676	0.002075	*Firmicutes*	*Clostridia*
DENOVO132	11.69	-5.87	1.86	-3.15	0.001637	0.004347	*Firmicutes*	*Clostridia*
DENOVO143	18.14	-7.03	2.30	-3.05	0.002252	0.005648	*Firmicutes*	*Clostridia*
DENOVO1054	4.59	-5.47	2.36	-2.32	0.020610	0.038132	*Firmicutes*	*Clostridia*
DENOVO55	10.53	-6.34	1.99	-3.19	0.001421	0.003857	*Firmicutes*	*Clostridia*
DENOVO116	6.77	-5.40	1.84	-2.94	0.003267	0.007760	*Firmicutes*	*Clostridia*
DENOVO356	26.73	-7.01	2.27	-3.08	0.002060	0.005299	*Firmicutes*	*Clostridia*
DENOVO1321	13.67	-6.53	2.39	-2.73	0.006401	0.013749	*Firmicutes*	*Clostridia*
DENOVO312	29.01	-7.66	2.31	-3.32	0.000898	0.002657	*Firmicutes*	*Clostridia*
DENOVO336	14.16	-7.09	2.06	-3.44	0.000591	0.001897	*Firmicutes*	*Clostridia*
DENOVO320	17.21	-7.03	2.29	-3.07	0.002136	0.005403	*Firmicutes*	*Clostridia*
DENOVO217	20.72	-7.61	2.08	-3.66	0.000254	0.000883	*Firmicutes*	*Clostridia*
DENOVO455	19.06	-7.61	1.98	-3.84	0.000125	0.000478	*Firmicutes*	*Clostridia*
DENOVO140	80.30	-8.99	2.24	-4.00	0.000062	0.000264	*Firmicutes*	*Clostridia*
DENOVO646	23.35	-7.41	2.30	-3.22	0.001262	0.003517	*Firmicutes*	*Clostridia*
DENOVO121	7.43	-6.15	2.14	-2.88	0.003954	0.009257	*Firmicutes*	*Clostridia*
DENOVO423	8.00	-5.86	2.14	-2.74	0.006083	0.013122	*Firmicutes*	*Clostridia*
DENOVO538	7.71	-5.98	1.91	-3.13	0.001774	0.004636	*Firmicutes*	*Clostridia*
DENOVO156	16.86	-6.95	1.74	-4.00	0.000062	0.000264	*Firmicutes*	*Clostridia*
DENOVO144	24.22	-8.28	1.66	-4.98	0.000001	0.000005	*Firmicutes*	*Clostridia*
DENOVO64	58.46	-5.34	1.36	-3.94	0.000082	0.000334	*Firmicutes*	*Clostridia*
DENOVO51	31.30	-7.55	2.32	-3.26	0.001106	0.003140	*Firmicutes*	*Clostridia*
DENOVO23	15.72	-6.78	1.74	-3.90	0.000097	0.000387	*Firmicutes*	*Clostridia*
DENOVO147	13.42	-6.51	2.47	-2.63	0.008445	0.017528	*Firmicutes*	*Clostridia*
DENOVO52	115.29	-8.95	2.28	-3.93	0.000086	0.000349	*Firmicutes*	*Clostridia*
DENOVO267	30.86	-8.25	1.86	-4.43	0.000010	0.000050	*Firmicutes*	*Clostridia*
DENOVO68	118.01	-10.05	1.84	-5.45	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO288	19.29	-7.45	2.09	-3.57	0.000356	0.001189	*Firmicutes*	*Clostridia*
DENOVO174	32.73	-7.96	2.21	-3.60	0.000324	0.001098	*Firmicutes*	*Clostridia*

DENOVO377	15.13	-6.63	2.47	-2.68	0.007320	0.015519	*Firmicutes*	*Clostridia*
DENOVO497	17.68	-7.10	2.29	-3.11	0.001900	0.004915	*Firmicutes*	*Clostridia*
DENOVO255	11.64	-6.73	2.06	-3.27	0.001076	0.003091	*Firmicutes*	*Clostridia*
DENOVO351	8.30	-4.98	2.14	-2.33	0.019881	0.037060	*Firmicutes*	*Clostridia*
DENOVO88	246.30	-10.74	1.92	-5.60	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO414	8.19	-5.90	2.37	-2.49	0.012689	0.025174	*Firmicutes*	*Clostridia*
DENOVO243	7.22	-5.78	2.36	-2.45	0.014283	0.027889	*Firmicutes*	*Clostridia*
DENOVO339	16.50	-7.03	2.28	-3.08	0.002079	0.005321	*Firmicutes*	*Clostridia*
DENOVO407	10.99	-6.60	1.96	-3.37	0.000744	0.002240	*Firmicutes*	*Clostridia*
DENOVO290	24.51	-7.59	2.20	-3.45	0.000552	0.001783	*Firmicutes*	*Clostridia*
DENOVO446	8.76	-6.11	1.93	-3.17	0.001541	0.004114	*Firmicutes*	*Clostridia*
DENOVO33	451.94	-11.37	1.97	-5.78	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO234	71.20	-8.90	2.09	-4.26	0.000021	0.000097	*Firmicutes*	*Clostridia*
DENOVO268	6.34	-5.84	2.36	-2.47	0.013447	0.026359	*Firmicutes*	*Clostridia*
DENOVO152	46.30	-8.32	2.23	-3.73	0.000190	0.000684	*Firmicutes*	*Clostridia*
DENOVO366	66.12	-9.04	2.05	-4.40	0.000011	0.000055	*Firmicutes*	*Clostridia*
DENOVO186	38.52	-8.45	2.02	-4.17	0.000030	0.000139	*Firmicutes*	*Clostridia*
DENOVO161	41.35	-8.61	1.92	-4.48	0.000008	0.000043	*Firmicutes*	*Clostridia*
DENOVO299	47.21	-8.55	2.13	-4.02	0.000058	0.000251	*Firmicutes*	*Clostridia*
DENOVO195	6.08	-5.45	2.40	-2.27	0.023120	0.041532	*Firmicutes*	*Clostridia*
DENOVO668	6.62	-4.86	2.14	-2.27	0.023001	0.041469	*Firmicutes*	*Clostridia*
DENOVO347	30.88	-8.27	1.92	-4.31	0.000016	0.000079	*Firmicutes*	*Clostridia*
DENOVO70	425.02	-11.15	1.71	-6.53	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO98	13.63	-7.10	1.89	-3.77	0.000166	0.000616	*Firmicutes*	*Clostridia*
DENOVO125	8.95	-4.93	1.87	-2.64	0.008270	0.017237	*Firmicutes*	*Clostridia*
DENOVO87	37.63	-8.74	1.67	-5.22	0.000000	0.000002	*Firmicutes*	*Clostridia*
DENOVO550	15.77	-7.15	2.16	-3.31	0.000944	0.002760	*Firmicutes*	*Clostridia*
DENOVO369	15.10	-7.16	2.07	-3.46	0.000539	0.001752	*Firmicutes*	*Clostridia*
DENOVO284	15.95	-7.30	2.06	-3.54	0.000400	0.001326	*Firmicutes*	*Clostridia*

DENOVO194	1.83	-4.07	1.84	-2.21	0.026915	0.047828	*Firmicutes*	*Clostridia*
DENOVO79	3.26	-5.40	2.21	-2.44	0.014544	0.028244	*Firmicutes*	*Clostridia*
DENOVO142	19.81	-6.95	2.47	-2.81	0.004916	0.010939	*Firmicutes*	*Clostridia*
DENOVO1230	1.51	-4.77	2.07	-2.30	0.021371	0.039247	*Firmicutes*	*Clostridia*
DENOVO302	17.68	-7.60	1.75	-4.34	0.000014	0.000070	*Firmicutes*	*Clostridia*
DENOVO220	66.05	-9.15	1.88	-4.87	0.000001	0.000007	*Firmicutes*	*Clostridia*
DENOVO432	18.03	-6.82	2.47	-2.76	0.005782	0.012583	*Firmicutes*	*Clostridia*
DENOVO315	6.50	-5.32	2.13	-2.50	0.012400	0.024700	*Firmicutes*	*Clostridia*
DENOVO385	3.62	-5.33	2.08	-2.56	0.010394	0.021044	*Firmicutes*	*Clostridia*
DENOVO239	9.20	-5.19	2.04	-2.54	0.010960	0.022098	*Firmicutes*	*Clostridia*
DENOVO115	5.89	-5.92	1.75	-3.39	0.000695	0.002119	*Firmicutes*	*Clostridia*
DENOVO647	21.39	-7.75	1.99	-3.89	0.000099	0.000393	*Firmicutes*	*Clostridia*
DENOVO995	9.68	-5.89	2.05	-2.87	0.004122	0.009560	*Firmicutes*	*Clostridia*
DENOVO141	121.87	-9.66	2.04	-4.75	0.000002	0.000013	*Firmicutes*	*Clostridia*
DENOVO308	12.81	-6.76	2.15	-3.14	0.001683	0.004445	*Firmicutes*	*Clostridia*
DENOVO218	13.26	-7.29	1.88	-3.89	0.000102	0.000401	*Firmicutes*	*Clostridia*
DENOVO1158	7.16	-5.41	2.36	-2.29	0.021738	0.039773	*Firmicutes*	*Clostridia*
DENOVO256	36.15	-8.27	2.09	-3.95	0.000077	0.000315	*Firmicutes*	*Clostridia*
DENOVO355	18.19	-7.52	1.89	-3.98	0.000068	0.000284	*Firmicutes*	*Clostridia*
DENOVO183	19.98	-6.96	2.47	-2.82	0.004854	0.010851	*Firmicutes*	*Clostridia*
DENOVO365	8.00	-5.90	1.92	-3.08	0.002093	0.005330	*Firmicutes*	*Clostridia*
DENOVO176	31.14	-7.74	2.31	-3.34	0.000827	0.002461	*Firmicutes*	*Clostridia*
DENOVO1188	33.99	-7.83	2.32	-3.38	0.000716	0.002171	*Firmicutes*	*Clostridia*
DENOVO311	29.94	-7.58	2.32	-3.27	0.001085	0.003098	*Firmicutes*	*Clostridia*
DENOVO295	8.83	-5.48	1.94	-2.82	0.004758	0.010684	*Firmicutes*	*Clostridia*
DENOVO406	3.85	-4.88	2.08	-2.34	0.019128	0.035929	*Firmicutes*	*Clostridia*
DENOVO334	21.42	-7.13	2.41	-2.96	0.003107	0.007486	*Firmicutes*	*Clostridia*
DENOVO769	44.80	-8.25	2.14	-3.86	0.000112	0.000432	*Firmicutes*	*Clostridia*
DENOVO35	85.57	-9.13	2.00	-4.56	0.000005	0.000030	*Firmicutes*	*Clostridia*

DENOVO661	104.54	-9.51	1.95	-4.88	0.000001	0.000007	*Firmicutes*	*Clostridia*
DENOVO82	10.82	-6.65	2.06	-3.22	0.001281	0.003535	*Firmicutes*	*Clostridia*
DENOVO470	3.70	-5.22	1.92	-2.72	0.006597	0.014109	*Firmicutes*	*Clostridia*
DENOVO44	193.67	-10.13	1.77	-5.74	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO1494	34.21	-8.33	2.01	-4.14	0.000035	0.000157	*Firmicutes*	*Clostridia*
DENOVO490	20.59	-7.49	2.09	-3.58	0.000343	0.001151	*Firmicutes*	*Clostridia*
DENOVO331	19.40	-7.36	2.18	-3.37	0.000748	0.002240	*Firmicutes*	*Clostridia*
DENOVO177	27.53	-7.54	2.32	-3.25	0.001148	0.003221	*Firmicutes*	*Clostridia*
DENOVO1102	11.20	-6.24	2.38	-2.62	0.008773	0.018134	*Firmicutes*	*Clostridia*
DENOVO562	10.74	-6.35	2.26	-2.80	0.005082	0.011259	*Firmicutes*	*Clostridia*
DENOVO36	562.11	-11.46	1.75	-6.56	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO338	29.32	-7.95	2.11	-3.77	0.000163	0.000611	*Firmicutes*	*Clostridia*
DENOVO649	63.32	-8.84	2.14	-4.13	0.000037	0.000165	*Firmicutes*	*Clostridia*
DENOVO14	666.49	-11.59	1.47	-7.89	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO148	77.66	-9.35	1.98	-4.73	0.000002	0.000014	*Firmicutes*	*Clostridia*
DENOVO926	6.79	-5.76	2.24	-2.58	0.009940	0.020290	*Firmicutes*	*Clostridia*
DENOVO884	5.00	-5.56	2.21	-2.51	0.011913	0.023827	*Firmicutes*	*Clostridia*
DENOVO27	163.45	-9.93	1.63	-6.11	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO535	16.52	-6.55	2.47	-2.65	0.008051	0.016924	*Firmicutes*	*Clostridia*
DENOVO136	250.58	-10.58	1.85	-5.72	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO473	11.63	-6.48	2.20	-2.95	0.003214	0.007708	*Firmicutes*	*Clostridia*
DENOVO1190	19.53	-7.03	2.41	-2.92	0.003502	0.008237	*Firmicutes*	*Clostridia*
DENOVO322	41.95	-8.07	2.32	-3.47	0.000515	0.001684	*Firmicutes*	*Clostridia*
DENOVO457	22.33	-7.37	2.30	-3.21	0.001337	0.003670	*Firmicutes*	*Clostridia*
DENOVO264	27.33	-7.87	2.10	-3.75	0.000180	0.000652	*Firmicutes*	*Clostridia*
DENOVO808	2.94	-5.42	2.09	-2.59	0.009632	0.019743	*Firmicutes*	*Bacilli*
DENOVO399	32.17	4.79	1.28	3.75	0.000174	0.000637	*Firmicutes*	*Bacilli*
DENOVO60	516.55	-4.46	0.65	-6.83	0.000000	0.000000	*Firmicutes*	*Bacilli*
DENOVO2150	2.32	-4.98	1.38	-3.61	0.000307	0.001045	*Firmicutes*	*Bacilli*

DENOVO310	8.04	-6.34	2.12	-2.99	0.002776	0.006789	*Firmicutes*	*Bacilli*
DENOVO18	1051.33	-7.31	0.96	-7.58	0.000000	0.000000	*Firmicutes*	*Bacilli*
DENOVO1372	31.13	-2.82	1.20	-2.35	0.018947	0.035725	*Firmicutes*	*Bacilli*
DENOVO5	11967.98	-3.44	1.17	-2.93	0.003357	0.007935	*Firmicutes*	*Bacilli*
DENOVO434	20.90	-5.04	1.58	-3.20	0.001387	0.003785	*Firmicutes*	*Bacilli*
DENOVO2157	2.57	-5.04	1.96	-2.57	0.010084	0.020499	*Firmicutes*	*Bacilli*
DENOVO372	40.90	-8.69	1.32	-6.59	0.000000	0.000000	*Firmicutes*	*Bacilli*
DENOVO208	29.67	-2.85	1.15	-2.48	0.013177	0.025934	*Firmicutes*	*Bacilli*
DENOVO102	84.49	-2.15	0.90	-2.39	0.016996	0.032669	*Firmicutes*	*Bacilli*
DENOVO207	32.93	-8.64	1.67	-5.18	0.000000	0.000002	*Proteobacteria*	*Deltaproteobacteria*
DENOVO187	75.34	-9.76	1.53	-6.38	0.000000	0.000000	*Proteobacteria*	*Deltaproteobacteria*
DENOVO190	19.72	-7.32	1.58	-4.64	0.000004	0.000022	*Proteobacteria*	*Deltaproteobacteria*
DENOVO1053	13.85	-7.41	1.70	-4.37	0.000012	0.000063	*Proteobacteria*	*Gammaproteobacteria*
DENOVO352	24.58	-3.99	1.73	-2.31	0.021148	0.038982	*Proteobacteria*	*Gammaproteobacteria*
DENOVO130	74.24	-7.25	1.40	-5.18	0.000000	0.000002	*Proteobacteria*	*Gammaproteobacteria*
DENOVO280	55.32	3.08	1.34	2.29	0.021921	0.039960	*Proteobacteria*	*Gammaproteobacteria*
DENOVO408	6.03	-6.43	1.88	-3.42	0.000635	0.002023	*Proteobacteria*	*Gammaproteobacteria*
DENOVO843	9.01	-5.58	1.76	-3.17	0.001517	0.004073	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1031	9.47	-6.63	2.13	-3.12	0.001818	0.004726	*Proteobacteria*	*Gammaproteobacteria*
DENOVO29	586.23	-11.62	1.11	-10.43	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO362	243.10	-10.96	1.47	-7.48	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO540	38.94	-8.41	2.02	-4.16	0.000032	0.000148	*Proteobacteria*	*Gammaproteobacteria*
DENOVO38	295.29	-8.00	1.22	-6.56	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO105	1015.80	-9.51	1.00	-9.47	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO522	16.30	-7.77	1.74	-4.47	0.000008	0.000043	*Proteobacteria*	*Gammaproteobacteria*
DENOVO12	113.74	-9.92	1.17	-8.45	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1	1170.78	3.06	0.60	5.11	0.000000	0.000003	*Proteobacteria*	*Gammaproteobacteria*
DENOVO754	2.41	-5.22	1.83	-2.85	0.004433	0.010139	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1249	2.78	-5.77	1.59	-3.62	0.000297	0.001017	*Proteobacteria*	*Gammaproteobacteria*

DENOVO154	18.30	-8.07	1.55	-5.22	0.000000	0.000002	*Proteobacteria*	*Gammaproteobacteria*
DENOVO103	133.48	-5.17	1.70	-3.05	0.002304	0.005748	*Proteobacteria*	*Gammaproteobacteria*
DENOVO393	24.32	-7.70	1.88	-4.09	0.000043	0.000188	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1088	10.68	-4.99	1.80	-2.78	0.005465	0.012052	*Proteobacteria*	*Gammaproteobacteria*
DENOVO392	31.32	-5.41	1.59	-3.41	0.000659	0.002047	*Proteobacteria*	*Gammaproteobacteria*
DENOVO828	9.21	-4.07	1.74	-2.33	0.019601	0.036678	*Proteobacteria*	*Gammaproteobacteria*
DENOVO386	37.68	-8.72	1.82	-4.80	0.000002	0.000010	*Proteobacteria*	*Betaproteobacteria*
DENOVO57	203.39	-6.57	1.42	-4.61	0.000004	0.000024	*Proteobacteria*	*Betaproteobacteria*
DENOVO642	3.12	-5.23	1.98	-2.64	0.008207	0.017179	*Proteobacteria*	*Betaproteobacteria*
DENOVO246	9.16	-4.61	1.89	-2.44	0.014579	0.028244	*Proteobacteria*	*Gammaproteobacteria*
DENOVO198	76.12	-7.56	1.39	-5.44	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO905	16.02	-6.34	1.92	-3.31	0.000943	0.002760	*Proteobacteria*	*Gammaproteobacteria*
DENOVO321	33.52	-6.00	1.62	-3.70	0.000213	0.000761	*Proteobacteria*	*Gammaproteobacteria*
DENOVO602	3.42	-5.23	1.77	-2.96	0.003066	0.007424	*Proteobacteria*	*Alphaproteobacteria*
DENOVO520	3.94	-5.56	1.52	-3.65	0.000262	0.000904	*Proteobacteria*	*Alphaproteobacteria*
DENOVO359	36.39	-4.85	1.42	-3.41	0.000640	0.002025	*Proteobacteria*	*Alphaproteobacteria*
DENOVO367	1.73	-4.10	1.73	-2.37	0.017979	0.034029	*Proteobacteria*	*Alphaproteobacteria*
DENOVO525	12.76	-6.04	2.28	-2.65	0.008025	0.016924	*Proteobacteria*	*Alphaproteobacteria*
DENOVO210	13.73	-6.72	2.27	-2.97	0.003022	0.007354	*Proteobacteria*	*Alphaproteobacteria*
DENOVO8	38.79	-8.45	1.48	-5.71	0.000000	0.000000	*Deferribacteres*	*Deferribacteres*
DENOVO16	4.89	-5.51	1.99	-2.78	0.005515	0.012109	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO15	2.18	-5.02	1.74	-2.88	0.004030	0.009392	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO10	526.24	-10.64	1.50	-7.09	0.000000	0.000000	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO578	5.22	-4.25	1.89	-2.25	0.024548	0.043779	*Actinobacteria*	*Actinobacteria*
DENOVO340	50.21	-9.26	1.29	-7.18	0.000000	0.000000	*Actinobacteria*	*Actinobacteria*
DENOVO241	55.96	-6.49	1.33	-4.87	0.000001	0.000007	*Actinobacteria*	*Actinobacteria*
DENOVO436	10.13	-6.32	1.94	-3.26	0.001114	0.003143	*Actinobacteria*	*Actinobacteria*
DENOVO511	6.32	-6.12	1.90	-3.22	0.001267	0.003517	*Actinobacteria*	*Actinobacteria*
DENOVO553	5.07	-5.00	2.21	-2.26	0.023706	0.042431	*Actinobacteria*	*Actinobacteria*

DENOVO291	17.11	-4.24	1.54	-2.75	0.006048	0.013104	*Actinobacteria*	*Actinobacteria*
DENOVO397	8.99	-6.86	1.65	-4.16	0.000032	0.000148	*Actinobacteria*	*Actinobacteria*
DENOVO471	34.20	-8.20	2.10	-3.91	0.000093	0.000372	*Actinobacteria*	*Actinobacteria*
DENOVO421	8.22	-6.81	1.51	-4.52	0.000006	0.000036	*Actinobacteria*	*Actinobacteria*
DENOVO508	3.79	-5.35	1.87	-2.85	0.004304	0.009890	*Actinobacteria*	*Actinobacteria*
DENOVO722	1.95	-4.30	1.94	-2.21	0.027134	0.048044	*Actinobacteria*	*Actinobacteria*
DENOVO433	11.47	-7.11	1.84	-3.87	0.000110	0.000426	*Actinobacteria*	*Actinobacteria*
DENOVO294	37.35	-8.74	1.75	-4.99	0.000001	0.000004	*Actinobacteria*	*Actinobacteria*
DENOVO1163	2.05	-4.66	1.96	-2.38	0.017371	0.033132	*Actinobacteria*	*Actinobacteria*
DENOVO1042	5.62	-5.92	1.89	-3.14	0.001699	0.004465	*Actinobacteria*	*Actinobacteria*
DENOVO444	3.93	-5.23	1.50	-3.50	0.000467	0.001538	*Actinobacteria*	*Actinobacteria*
DENOVO191	45.96	-3.57	1.57	-2.27	0.022934	0.041469	*Actinobacteria*	*Actinobacteria*
DENOVO209	8.32	-5.57	1.96	-2.84	0.004574	0.010317	*Actinobacteria*	*Actinobacteria*
DENOVO465	7.74	-6.11	2.35	-2.60	0.009446	0.019444	*Bacteroidetes*	*Bacteroidia*
DENOVO235	5.54	-5.92	1.97	-3.00	0.002741	0.006736	*Bacteroidetes*	*Bacteroidia*
DENOVO428	21.06	-7.46	2.19	-3.40	0.000664	0.002049	*Bacteroidetes*	*Bacteroidia*
DENOVO24	777.68	-11.97	1.90	-6.31	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO40	91.84	-9.36	2.02	-4.64	0.000004	0.000022	*Bacteroidetes*	*Bacteroidia*
DENOVO309	11.27	-6.64	2.16	-3.07	0.002144	0.005403	*Bacteroidetes*	*Bacteroidia*
DENOVO17	172.33	-10.39	1.85	-5.60	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO89	53.15	-8.10	2.46	-3.29	0.000985	0.002862	*Bacteroidetes*	*Bacteroidia*
DENOVO53	58.86	-9.00	1.76	-5.12	0.000000	0.000002	*Bacteroidetes*	*Bacteroidia*
DENOVO230	28.54	-7.96	1.83	-4.36	0.000013	0.000064	*Bacteroidetes*	*Bacteroidia*
DENOVO84	107.21	-8.87	2.20	-4.04	0.000053	0.000231	*Bacteroidetes*	*Bacteroidia*
DENOVO170	25.75	-8.01	1.83	-4.37	0.000012	0.000062	*Bacteroidetes*	*Bacteroidia*
DENOVO129	7.66	-6.40	2.12	-3.02	0.002569	0.006344	*Bacteroidetes*	*Bacteroidia*
DENOVO541	9.09	-6.19	2.04	-3.03	0.002408	0.005979	*Bacteroidetes*	*Bacteroidia*
DENOVO22	1524.80	-11.97	1.59	-7.54	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO163	24.69	-7.99	1.96	-4.07	0.000047	0.000205	*Bacteroidetes*	*Bacteroidia*

DENOVO197	52.21	-8.71	2.11	-4.13	0.000037	0.000165	*Bacteroidetes*	*Bacteroidia*
DENOVO219	39.31	-8.50	1.86	-4.57	0.000005	0.000028	*Bacteroidetes*	*Bacteroidia*
DENOVO48	941.01	-13.25	1.57	-8.42	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO205	214.24	-10.99	1.76	-6.25	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO74	1044.66	-12.92	1.64	-7.87	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO188	84.99	-9.42	1.92	-4.91	0.000001	0.000006	*Bacteroidetes*	*Bacteroidia*
DENOVO138	207.21	-10.78	1.78	-6.06	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO164	56.91	-9.12	1.77	-5.14	0.000000	0.000002	*Bacteroidetes*	*Bacteroidia*
DENOVO258	67.19	-7.85	1.96	-4.00	0.000063	0.000264	*Bacteroidetes*	*Bacteroidia*
DENOVO160	3.82	-5.41	2.34	-2.32	0.020553	0.038132	*Bacteroidetes*	*Bacteroidia*
DENOVO104	285.76	-8.05	1.60	-5.02	0.000001	0.000004	*Bacteroidetes*	*Bacteroidia*
DENOVO357	34.85	-8.32	1.75	-4.76	0.000002	0.000012	*Bacteroidetes*	*Bacteroidia*
DENOVO472	7.34	-6.24	1.90	-3.28	0.001037	0.002997	*Bacteroidetes*	*Bacteroidia*
DENOVO66	572.63	-12.42	1.70	-7.29	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO252	83.06	-9.54	1.95	-4.90	0.000001	0.000007	*Bacteroidetes*	*Bacteroidia*
DENOVO390	21.66	-7.33	2.30	-3.19	0.001434	0.003870	*Bacteroidetes*	*Bacteroidia*
DENOVO146	80.58	-10.14	1.50	-6.76	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO117	829.69	-11.70	1.92	-6.09	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO150	194.53	-10.94	1.75	-6.24	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO100	500.42	-11.71	1.97	-5.95	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO31	163.81	-10.21	1.41	-7.26	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO237	49.65	-8.70	1.95	-4.47	0.000008	0.000043	*Bacteroidetes*	*Bacteroidia*
DENOVO96	196.87	-10.38	1.35	-7.69	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO306	8.23	-6.34	2.24	-2.84	0.004571	0.010317	*Bacteroidetes*	*Bacteroidia*
DENOVO391	7.97	-6.47	1.90	-3.41	0.000655	0.002047	*Bacteroidetes*	*Bacteroidia*
DENOVO165	213.21	-11.16	1.68	-6.63	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO58	416.60	-12.26	1.49	-8.23	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO251	8.62	-5.85	2.05	-2.86	0.004227	0.009759	*Bacteroidetes*	*Bacteroidia*
DENOVO269	58.83	-9.67	1.54	-6.27	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*

DENOVO526	29.44	-8.01	1.81	-4.42	0.000010	0.000052	*Bacteroidetes*	*Bacteroidia*
DENOVO486	3.50	-5.32	2.33	-2.28	0.022586	0.041021	*Bacteroidetes*	*Bacteroidia*
DENOVO222	11.32	-6.65	2.26	-2.94	0.003245	0.007744	*Bacteroidetes*	*Bacteroidia*
DENOVO279	64.93	-9.49	1.77	-5.36	0.000000	0.000001	*Bacteroidetes*	*Bacteroidia*
DENOVO101	227.41	-11.33	1.38	-8.19	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO83	548.29	-12.19	1.80	-6.76	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO47	115.47	-6.89	1.56	-4.43	0.000009	0.000050	*Bacteroidetes*	*Bacteroidia*
DENOVO467	126.97	-10.56	1.53	-6.89	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO81	232.59	-10.57	1.76	-6.00	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO242	12.02	-7.12	1.95	-3.66	0.000253	0.000883	*Bacteroidetes*	*Bacteroidia*
DENOVO134	63.78	-9.46	1.77	-5.34	0.000000	0.000001	*Bacteroidetes*	*Bacteroidia*
DENOVO107	52.68	-8.87	1.82	-4.88	0.000001	0.000007	*Bacteroidetes*	*Bacteroidia*
DENOVO56	322.79	-11.44	1.48	-7.74	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO63	52.85	-8.73	1.95	-4.47	0.000008	0.000043	*Bacteroidetes*	*Bacteroidia*
DENOVO50	200.30	-10.44	2.06	-5.06	0.000000	0.000003	*Bacteroidetes*	*Bacteroidia*
DENOVO270	15.24	-7.36	1.96	-3.76	0.000169	0.000623	*Bacteroidetes*	*Bacteroidia*
DENOVO286	1.08	-4.64	1.96	-2.37	0.017706	0.033642	*Bacteroidetes*	*Bacteroidia*
DENOVO30	222.94	-11.29	1.39	-8.13	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO184	4.89	-5.95	2.10	-2.84	0.004544	0.010317	*Bacteroidetes*	*Bacteroidia*
DENOVO114	458.41	-11.76	1.81	-6.51	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO225	28.78	-7.99	2.09	-3.82	0.000131	0.000497	*Bacteroidetes*	*Bacteroidia*
DENOVO109	268.12	-11.58	1.45	-8.00	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO166	42.88	-8.56	2.01	-4.26	0.000021	0.000097	*Bacteroidetes*	*Bacteroidia*
DENOVO93	44.29	-8.52	1.28	-6.66	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO73	653.48	-12.18	1.56	-7.82	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO46	694.35	-12.59	1.37	-9.21	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO257	18.07	-7.84	1.76	-4.44	0.000009	0.000048	*Bacteroidetes*	*Bacteroidia*
DENOVO158	29.59	-8.26	1.90	-4.34	0.000014	0.000070	*Bacteroidetes*	*Bacteroidia*
DENOVO19	405.81	-12.02	1.18	-10.18	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*

DENOVO11	2511.80	-10.89	1.45	-7.54	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO45	160.19	-7.29	1.41	-5.16	0.000000	0.000002	*Bacteroidetes*	*Bacteroidia*
DENOVO120	171.81	-11.13	1.47	-7.55	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO296	7.58	-6.01	2.38	-2.53	0.011403	0.022899	*Bacteroidetes*	*Bacteroidia*
DENOVO39	30.98	-8.07	1.74	-4.64	0.000004	0.000022	*Bacteroidetes*	*Bacteroidia*
DENOVO34	14.85	-6.09	1.61	-3.77	0.000161	0.000606	*Bacteroidetes*	*Bacteroidia*
DENOVO26	31.71	-7.97	1.75	-4.56	0.000005	0.000030	*Bacteroidetes*	*Bacteroidia*
DENOVO49	4.76	-5.35	2.23	-2.40	0.016197	0.031256	*Bacteroidetes*	*Bacteroidia*
DENOVO7	0.98	-3.31	1.39	-2.38	0.017313	0.033132	*Tenericutes*	*Mollicutes*
DENOVO4	1.11	-2.71	1.09	-2.48	0.013117	0.025918	*Tenericutes*	*Mollicutes*
DENOVO61	359.08	-11.25	1.48	-7.61	0.000000	0.000000	*Candidatus Saccharibacteria*	*Saccharibacteria*

Table A.6.3: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the small intestine and *Aonchotheca murissylvatici.*

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	p-value	Adjusted p-value	Phylum	Class
DENOVO245	24.53	8.78	2.22	3.96	0.000074	0.011338	*Proteobacteria*	*Betaproteobacteria*
DENOVO249	91.00	8.08	2.27	3.57	0.000363	0.045628	*Proteobacteria*	*Betaproteobacteria*
DENOVO394	36.87	8.29	2.09	3.96	0.000075	0.011338	*Proteobacteria*	*Alphaproteobacteria*
DENOVO515	94.72	10.33	1.91	5.42	0.000000	0.000023	*Bacteroidetes*	*Flavobacteriia*
DENOVO1348	58.05	9.93	1.90	5.23	0.000000	0.000042	*Bacteroidetes*	*Flavobacteriia*
DENOVO3	125861.92	13.58	2.20	6.17	0.000000	0.000001	*Tenericutes*	*Mollicutes*

Table A.6.4: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the small intestine and *Heligmosomoides polygyrus.*

OTU	Base Mean	Log^2 fold change	Log^2 fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO60	41.25	-2.84	0.83	-3.42	0.000636	0.009958	*Bacilli*	*Lactobacillales*
DENOVO247	8.45	6.34	2.22	2.86	0.004229	0.046311	*Bacilli*	*Lactobacillales*
DENOVO204	65.02	7.81	2.31	3.38	0.000738	0.010332	*Bacilli*	*Lactobacillales*
DENOVO208	33.15	5.27	1.09	4.83	0.000001	0.000052	*Bacilli*	*Bacillales*
DENOVO438	5.47	6.30	1.63	3.87	0.000111	0.002673	*Bacilli*	*Bacillales*
DENOVO102	17.32	-3.42	0.94	-3.63	0.000289	0.005340	*Bacilli*	*Bacillales*
DENOVO189	15.86	7.28	1.19	6.14	0.000000	0.000000	*Gammaproteobacteria*	*Xanthomonadales*
DENOVO1	4978.04	3.89	1.08	3.61	0.000301	0.005340	*Gammaproteobacteria*	*Enterobacteriales*
DENOVO614	4.39	5.80	1.99	2.92	0.003494	0.042243	*Gammaproteobacteria*	*Pseudomonadales*
DENOVO656	11.00	6.56	1.93	3.40	0.000682	0.010075	*Betaproteobacteria*	*Burkholderiales*
DENOVO493	6.53	6.18	1.99	3.11	0.001852	0.024629	*Betaproteobacteria*	*Burkholderiales*
DENOVO245	68.01	9.57	1.40	6.85	0.000000	0.000000	*Betaproteobacteria*	*Burkholderiales*
DENOVO249	60.54	8.97	1.23	7.28	0.000000	0.000000	*Betaproteobacteria*	*Burkholderiales*
DENOVO667	11.71	6.89	1.83	3.78	0.000160	0.003539	*Betaproteobacteria*	*Burkholderiales*
DENOVO573	4.82	6.10	1.98	3.08	0.002077	0.026313	*Betaproteobacteria*	*Methylophilales*
DENOVO394	3.06	5.46	1.49	3.67	0.000244	0.004984	*Alphaproteobacteria*	*Rhizobiales*
DENOVO180	65.16	5.10	1.14	4.47	0.000008	0.000234	*Alphaproteobacteria*	*Rhizobiales*
DENOVO153	47.51	5.40	1.37	3.95	0.000079	0.002101	*Alphaproteobacteria*	*Rhizobiales*
DENOVO977	3.88	6.00	1.68	3.57	0.000354	0.005879	*Alphaproteobacteria*	*Sphingomonadales*
DENOVO229	99.90	9.83	1.23	7.97	0.000000	0.000000	*Alphaproteobacteria*	*Sphingomonadales*
DENOVO631	5.51	5.60	1.96	2.85	0.004353	0.046311	*Alphaproteobacteria*	*Rhodobacterales*
DENOVO1486	6.19	5.75	1.99	2.89	0.003909	0.045213	*Actinobacteria*	*Actinomycetales*

DENOVO505	9.37	7.26	1.62	4.48	0.000008	0.000234	*Actinobacteria*	*Actinomycetales*
DENOVO9	29000.89	10.33	1.44	7.15	0.000000	0.000000	*Mollicutes*	*Mycoplasmatales*
DENOVO3	338.13	8.27	1.66	4.98	0.000001	0.000029	*Mollicutes*	*Mycoplasmatales*

Table A.6.5: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the small intestine and *Hymenolepis diminuta*.

OTU	Base Mean	Log^2 fold change	Log^2 fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO86	0.76	-3.32	1.04	-3.18	0.001469	0.004431	*Firmicutes*	*Clostridia*
DENOVO28	0.48	-2.82	0.94	-3.01	0.002654	0.007540	*Firmicutes*	*Clostridia*
DENOVO112	0.75	-3.49	1.18	-2.95	0.003175	0.008889	*Firmicutes*	*Clostridia*
DENOVO144	0.91	-3.70	0.91	-4.05	0.000052	0.000249	*Firmicutes*	*Clostridia*
DENOVO64	0.65	-2.57	1.01	-2.54	0.011083	0.025860	*Firmicutes*	*Clostridia*
DENOVO51	1.25	-3.97	0.98	-4.05	0.000050	0.000246	*Firmicutes*	*Clostridia*
DENOVO23	4.78	-4.84	0.79	-6.15	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO52	2.51	-4.62	0.87	-5.30	0.000000	0.000001	*Firmicutes*	*Clostridia*
DENOVO68	1.89	-3.59	1.01	-3.54	0.000402	0.001460	*Firmicutes*	*Clostridia*
DENOVO33	2.03	-4.46	0.99	-4.49	0.000007	0.000044	*Firmicutes*	*Clostridia*
DENOVO169	4.12	-5.27	1.49	-3.55	0.000386	0.001428	*Firmicutes*	*Clostridia*
DENOVO195	0.65	-3.03	1.25	-2.42	0.015454	0.034033	*Firmicutes*	*Clostridia*
DENOVO70	0.65	-3.15	1.29	-2.44	0.014778	0.032914	*Firmicutes*	*Clostridia*
DENOVO98	1.06	-3.54	1.56	-2.27	0.023195	0.047356	*Firmicutes*	*Clostridia*
DENOVO87	1.16	-3.65	0.96	-3.81	0.000141	0.000615	*Firmicutes*	*Clostridia*
DENOVO220	0.45	-2.63	0.89	-2.94	0.003249	0.008969	*Firmicutes*	*Clostridia*
DENOVO72	45.41	-6.59	1.31	-5.03	0.000000	0.000005	*Firmicutes*	*Clostridia*
DENOVO44	1.57	-3.12	0.86	-3.62	0.000292	0.001100	*Firmicutes*	*Clostridia*

DENOVO36	1.68	-3.41	0.90	-3.81	0.000140	0.000615	*Firmicutes*	*Clostridia*
DENOVO14	4.39	-3.58	0.80	-4.50	0.000007	0.000044	*Firmicutes*	*Clostridia*
DENOVO27	2.76	-3.55	0.94	-3.79	0.000151	0.000643	*Firmicutes*	*Clostridia*
DENOVO404	0.88	3.54	1.12	3.15	0.001649	0.004753	*Firmicutes*	*Bacilli*
DENOVO719	0.96	3.84	1.04	3.68	0.000234	0.000919	*Firmicutes*	*Bacilli*
DENOVO193	11.17	-5.46	0.81	-6.74	0.000000	0.000000	*Firmicutes*	*Bacilli*
DENOVO60	24.18	-4.77	0.85	-5.62	0.000000	0.000000	*Firmicutes*	*Bacilli*
DENOVO18	87.81	-6.50	1.05	-6.17	0.000000	0.000000	*Firmicutes*	*Bacilli*
DENOVO301	1.97	-3.95	1.07	-3.69	0.000222	0.000888	*Firmicutes*	*Bacilli*
DENOVO6	135.65	-3.48	0.75	-4.64	0.000004	0.000024	*Firmicutes*	*Bacilli*
DENOVO5	167.39	-3.94	0.68	-5.83	0.000000	0.000000	*Firmicutes*	*Bacilli*
DENOVO2	1399.48	-2.11	0.62	-3.41	0.000646	0.002148	*Firmicutes*	*Bacilli*
DENOVO434	0.50	-2.91	1.26	-2.31	0.020724	0.044150	*Firmicutes*	*Bacilli*
DENOVO372	2.47	-3.05	0.96	-3.18	0.001449	0.004431	*Firmicutes*	*Bacilli*
DENOVO102	22.25	-6.82	0.84	-8.08	0.000000	0.000000	*Firmicutes*	*Bacilli*
DENOVO187	1.31	-3.36	1.01	-3.31	0.000924	0.002920	*Proteobacteria*	*Deltaproteobacteria*
DENOVO190	0.69	-2.92	1.10	-2.65	0.008127	0.019427	*Proteobacteria*	*Deltaproteobacteria*
DENOVO189	6.50	5.55	0.75	7.39	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO65	1.75	-3.82	0.85	-4.49	0.000007	0.000044	*Proteobacteria*	*Gammaproteobacteria*
DENOVO29	5.95	-5.48	0.95	-5.74	0.000000	0.000000	*Proteobacteria*	*Gammaproteobacteria*
DENOVO362	4.73	-5.54	1.35	-4.09	0.000043	0.000215	*Proteobacteria*	*Gammaproteobacteria*
DENOVO105	2.84	-3.81	1.03	-3.70	0.000217	0.000885	*Proteobacteria*	*Gammaproteobacteria*
DENOVO1	3892.42	2.51	0.73	3.45	0.000552	0.001932	*Proteobacteria*	*Gammaproteobacteria*
DENOVO249	5.27	4.94	0.75	6.60	0.000000	0.000000	*Proteobacteria*	*Betaproteobacteria*
DENOVO57	5.54	-4.39	0.84	-5.22	0.000000	0.000002	*Proteobacteria*	*Betaproteobacteria*
DENOVO520	0.56	-3.04	1.04	-2.92	0.003458	0.009413	*Proteobacteria*	*Alphaproteobacteria*
DENOVO394	0.79	3.32	0.95	3.48	0.000494	0.001761	*Proteobacteria*	*Alphaproteobacteria*
DENOVO180	3.63	2.83	0.60	4.69	0.000003	0.000020	*Proteobacteria*	*Alphaproteobacteria*
DENOVO773	0.50	-2.99	1.11	-2.69	0.007069	0.017319	*Proteobacteria*	*Alphaproteobacteria*

DENOVO229	1.85	3.27	0.84	3.90	0.000097	0.000453	*Proteobacteria*	*Alphaproteobacteria*
DENOVO8	6.33	-4.51	0.84	-5.39	0.000000	0.000001	*Deferribacteres*	*Deferribacteres*
DENOVO16	0.63	-2.75	1.11	-2.47	0.013677	0.030968	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO15	104.13	-9.49	1.11	-8.54	0.000000	0.000000	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO10	6.86	-4.95	0.75	-6.60	0.000000	0.000000	*Proteobacteria*	*Epsilonproteobacteria*
DENOVO241	1.03	-3.61	0.76	-4.72	0.000002	0.000018	*Actinobacteria*	*Actinobacteria*
DENOVO291	1.29	-3.50	1.10	-3.19	0.001422	0.004423	*Actinobacteria*	*Actinobacteria*
DENOVO444	0.46	-2.93	0.93	-3.17	0.001547	0.004580	*Actinobacteria*	*Actinobacteria*
DENOVO191	1.70	-4.01	1.05	-3.83	0.000128	0.000583	*Actinobacteria*	*Actinobacteria*
DENOVO794	0.32	-2.36	1.01	-2.34	0.019164	0.041353	*Actinobacteria*	*Actinobacteria*
DENOVO22	10.19	-5.06	1.02	-4.97	0.000001	0.000006	*Bacteroidetes*	*Bacteroidia*
DENOVO48	2.85	-4.10	1.19	-3.45	0.000564	0.001938	*Bacteroidetes*	*Bacteroidia*
DENOVO205	1.08	-3.64	1.08	-3.38	0.000737	0.002367	*Bacteroidetes*	*Bacteroidia*
DENOVO74	7.27	-5.13	0.97	-5.28	0.000000	0.000001	*Bacteroidetes*	*Bacteroidia*
DENOVO188	1.14	-3.13	1.37	-2.28	0.022612	0.047148	*Bacteroidetes*	*Bacteroidia*
DENOVO138	1.76	-2.83	1.25	-2.27	0.022997	0.047356	*Bacteroidetes*	*Bacteroidia*
DENOVO164	0.87	-2.92	1.09	-2.67	0.007621	0.018441	*Bacteroidetes*	*Bacteroidia*
DENOVO104	2.90	-4.10	0.94	-4.36	0.000013	0.000074	*Bacteroidetes*	*Bacteroidia*
DENOVO66	7.60	-4.17	1.11	-3.76	0.000172	0.000719	*Bacteroidetes*	*Bacteroidia*
DENOVO117	1.84	-2.69	1.08	-2.49	0.012935	0.029826	*Bacteroidetes*	*Bacteroidia*
DENOVO31	7.54	-3.87	0.89	-4.36	0.000013	0.000074	*Bacteroidetes*	*Bacteroidia*
DENOVO237	0.75	-2.62	1.15	-2.28	0.022516	0.047148	*Bacteroidetes*	*Bacteroidia*
DENOVO96	5.49	-3.90	0.87	-4.47	0.000008	0.000047	*Bacteroidetes*	*Bacteroidia*
DENOVO165	0.59	-2.86	1.06	-2.69	0.007039	0.017319	*Bacteroidetes*	*Bacteroidia*
DENOVO58	6.21	-4.66	0.96	-4.85	0.000001	0.000010	*Bacteroidetes*	*Bacteroidia*
DENOVO269	0.56	-2.63	0.97	-2.71	0.006690	0.017028	*Bacteroidetes*	*Bacteroidia*
DENOVO279	0.35	-2.55	1.03	-2.46	0.013746	0.030968	*Bacteroidetes*	*Bacteroidia*
DENOVO101	3.99	-4.29	0.92	-4.65	0.000003	0.000023	*Bacteroidetes*	*Bacteroidia*
DENOVO83	4.91	-4.20	1.24	-3.39	0.000693	0.002264	*Bacteroidetes*	*Bacteroidia*

DENOVO47	2.83	-3.22	1.19	-2.71	0.006667	0.017028	*Bacteroidetes*	*Bacteroidia*
DENOVO56	0.73	-2.75	0.98	-2.79	0.005216	0.013816	*Bacteroidetes*	*Bacteroidia*
DENOVO30	4.59	-5.23	0.91	-5.75	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO114	1.76	-3.79	1.10	-3.44	0.000581	0.001964	*Bacteroidetes*	*Bacteroidia*
DENOVO109	0.85	-2.71	0.97	-2.78	0.005380	0.014059	*Bacteroidetes*	*Bacteroidia*
DENOVO166	2.80	-3.17	1.17	-2.70	0.006851	0.017216	*Bacteroidetes*	*Bacteroidia*
DENOVO93	2.17	-4.11	0.99	-4.14	0.000034	0.000176	*Bacteroidetes*	*Bacteroidia*
DENOVO73	6.90	-5.33	0.96	-5.54	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO46	6.89	-3.87	0.91	-4.25	0.000022	0.000118	*Bacteroidetes*	*Bacteroidia*
DENOVO158	1.68	-3.07	1.31	-2.34	0.019199	0.041353	*Bacteroidetes*	*Bacteroidia*
DENOVO19	16.89	-4.71	0.77	-6.16	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO11	32.53	-3.97	0.94	-4.23	0.000023	0.000124	*Bacteroidetes*	*Bacteroidia*
DENOVO45	5.79	-4.33	0.85	-5.10	0.000000	0.000003	*Bacteroidetes*	*Bacteroidia*
DENOVO120	1.80	-4.09	0.85	-4.83	0.000001	0.000011	*Bacteroidetes*	*Bacteroidia*
DENOVO39	0.68	-2.70	1.03	-2.61	0.009122	0.021542	*Bacteroidetes*	*Bacteroidia*
DENOVO9	2.27	-2.88	0.91	-3.16	0.001565	0.004580	*Tenericutes*	*Mollicutes*
DENOVO7	18.62	-3.50	1.23	-2.84	0.004482	0.012033	*Tenericutes*	*Mollicutes*
DENOVO3	3564.85	7.18	1.04	6.91	0.000000	0.000000	*Tenericutes*	*Mollicutes*
DENOVO4	2339.22	5.16	1.06	4.87	0.000001	0.000010	*Tenericutes*	*Mollicutes*
DENOVO61	5.98	-3.68	1.01	-3.63	0.000278	0.001069	*Candidatus Saccharibacteria*	*Saccharibacteria*

Table A.6.6: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the caecum and *Aonchotheca murissylvatici.*

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class

DENOVO28	113.65	-7.16	2.43	-2.95	0.003145	0.036559	*Firmicutes*	*Clostridia*
DENOVO140	55.01	-6.86	2.45	-2.80	0.005176	0.046395	*Firmicutes*	*Clostridia*
DENOVO144	40.64	-6.80	2.38	-2.86	0.004176	0.041143	*Firmicutes*	*Clostridia*
DENOVO64	141.21	-7.38	2.40	-3.07	0.002127	0.035992	*Firmicutes*	*Clostridia*
DENOVO137	68.21	-7.10	2.43	-2.93	0.003409	0.036559	*Firmicutes*	*Clostridia*
DENOVO161	42.40	-6.73	2.46	-2.74	0.006192	0.048894	*Firmicutes*	*Clostridia*
DENOVO220	36.11	-6.61	2.41	-2.74	0.006226	0.048894	*Firmicutes*	*Clostridia*
DENOVO176	68.77	-7.09	2.43	-2.92	0.003482	0.036559	*Firmicutes*	*Clostridia*
DENOVO182	59.29	9.82	3.07	3.20	0.001358	0.035992	*Firmicutes*	*Clostridia*
DENOVO27	485.57	-9.39	2.20	-4.26	0.000020	0.005135	*Firmicutes*	*Clostridia*
DENOVO575	9.42	9.98	3.19	3.12	0.001790	0.035992	*Proteobacteria*	*Alphaproteobacteria*
DENOVO8	569.66	-7.00	2.45	-2.86	0.004262	0.041143	*Deferribacteres*	*Deferribacteres*
DENOVO17	287.99	-6.07	2.19	-2.78	0.005462	0.047271	*Bacteroidetes*	*Bacteroidia*
DENOVO22	183.55	-7.80	2.36	-3.31	0.000940	0.033696	*Bacteroidetes*	*Bacteroidia*
DENOVO74	51.58	-7.08	2.39	-2.96	0.003042	0.036559	*Bacteroidetes*	*Bacteroidia*
DENOVO66	99.86	-7.34	2.40	-3.05	0.002265	0.035992	*Bacteroidetes*	*Bacteroidia*
DENOVO31	72.78	-7.40	2.39	-3.09	0.001984	0.035992	*Bacteroidetes*	*Bacteroidia*
DENOVO96	41.91	-7.01	2.26	-3.10	0.001922	0.035992	*Bacteroidetes*	*Bacteroidia*
DENOVO58	82.62	-7.27	2.41	-3.02	0.002562	0.035992	*Bacteroidetes*	*Bacteroidia*
DENOVO269	18.31	-6.03	2.21	-2.74	0.006233	0.048894	*Bacteroidetes*	*Bacteroidia*
DENOVO101	61.15	-7.25	2.41	-3.01	0.002581	0.035992	*Bacteroidetes*	*Bacteroidia*
DENOVO81	39.36	-6.74	2.40	-2.81	0.004997	0.046395	*Bacteroidetes*	*Bacteroidia*
DENOVO134	32.79	-6.70	2.26	-2.96	0.003081	0.036559	*Bacteroidetes*	*Bacteroidia*
DENOVO56	68.13	-7.45	2.37	-3.14	0.001679	0.035992	*Bacteroidetes*	*Bacteroidia*
DENOVO109	68.32	-7.09	2.43	-2.92	0.003496	0.036559	*Bacteroidetes*	*Bacteroidia*
DENOVO73	73.99	-7.27	2.41	-3.02	0.002537	0.035992	*Bacteroidetes*	*Bacteroidia*
DENOVO19	193.23	-8.69	2.26	-3.84	0.000123	0.015378	*Bacteroidetes*	*Bacteroidia*
DENOVO45	86.50	-7.94	2.23	-3.57	0.000358	0.022463	*Bacteroidetes*	*Bacteroidia*
DENOVO120	40.56	-6.96	2.26	-3.07	0.002120	0.035992	*Bacteroidetes*	*Bacteroidia*

DENOVO39	288.73	-8.06	2.33	-3.45	0.000551	0.026783	*Bacteroidetes*	*Bacteroidia*
DENOVO345	36.81	11.41	3.19	3.57	0.000353	0.022463	*Cyanobacteria*	*Cyanobacteria*
DENOVO61	112.73	-7.96	2.33	-3.41	0.000640	0.026783	*Candidatus Saccharibacteria*	*Saccharibacteria*

Table A.6.7: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the caecum and *Hymenolepis diminuta.*

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO140	62.99	-6.01	1.82	-3.30	0.000979	0.022099	*Firmicutes*	*Clostridia*
DENOVO121	297.50	9.99	2.62	3.81	0.000141	0.006538	*Firmicutes*	*Clostridia*
DENOVO64	183.12	-6.75	1.90	-3.56	0.000372	0.012921	*Firmicutes*	*Clostridia*
DENOVO23	1997.34	5.45	1.85	2.95	0.003224	0.048357	*Firmicutes*	*Clostridia*
DENOVO341	54.23	10.55	2.73	3.86	0.000114	0.006348	*Firmicutes*	*Clostridia*
DENOVO661	166.89	-6.18	2.11	-2.93	0.003412	0.048357	*Firmicutes*	*Clostridia*
DENOVO372	46.94	7.49	2.42	3.09	0.001981	0.033479	*Firmicutes*	*Bacilli*
DENOVO207	40.87	-5.44	1.86	-2.92	0.003479	0.048357	*Proteobacteria*	*Deltaproteobacteria*
DENOVO209	29.93	8.55	2.62	3.26	0.001102	0.022099	*Actinobacteria*	*Actinobacteria*
DENOVO17	307.96	-7.47	1.90	-3.93	0.000084	0.006348	*Bacteroidetes*	*Bacteroidia*
DENOVO74	80.33	-6.15	1.87	-3.29	0.000993	0.022099	*Bacteroidetes*	*Bacteroidia*
DENOVO146	30.01	-5.39	1.75	-3.08	0.002047	0.033479	*Bacteroidetes*	*Bacteroidia*
DENOVO269	41.27	-5.63	1.79	-3.15	0.001615	0.029935	*Bacteroidetes*	*Bacteroidia*
DENOVO101	108.47	-5.98	1.83	-3.27	0.001073	0.022099	*Bacteroidetes*	*Bacteroidia*
DENOVO134	56.18	-6.12	1.74	-3.53	0.000421	0.013003	*Bacteroidetes*	*Bacteroidia*
DENOVO56	85.76	-6.45	1.78	-3.62	0.000300	0.011901	*Bacteroidetes*	*Bacteroidia*
DENOVO109	90.96	-6.19	1.90	-3.26	0.001113	0.022099	*Bacteroidetes*	*Bacteroidia*

DENOVO19	472.63	-7.25	1.81	-4.01	0.000062	0.006348	*Bacteroidetes*	*Bacteroidia*
DENOVO120	49.37	-6.29	1.62	-3.89	0.000101	0.006348	*Bacteroidetes*	*Bacteroidia*
DENOVO39	579.36	-7.78	2.00	-3.89	0.000100	0.006348	*Bacteroidetes*	*Bacteroidia*

Table A.6.8: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the caecum and *Syphacia frederici.*

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO484	7.88	-2.46	0.81	-3.03	0.002431	0.014199	*Firmicutes*	*Clostridia*
DENOVO533	28.96	-3.45	0.71	-4.85	0.000001	0.000047	*Firmicutes*	*Clostridia*
DENOVO95	16.10	-3.59	1.04	-3.47	0.000528	0.004717	*Firmicutes*	*Clostridia*
DENOVO132	24.94	-2.59	0.98	-2.65	0.008131	0.037710	*Firmicutes*	*Clostridia*
DENOVO1321	2.16	-3.17	1.24	-2.56	0.010525	0.045404	*Firmicutes*	*Clostridia*
DENOVO140	100.28	2.08	0.62	3.32	0.000886	0.006848	*Firmicutes*	*Clostridia*
DENOVO423	2.55	-2.12	0.81	-2.63	0.008543	0.039131	*Firmicutes*	*Clostridia*
DENOVO156	68.61	2.38	0.81	2.95	0.003216	0.017548	*Firmicutes*	*Clostridia*
DENOVO68	90.92	-3.36	0.67	-5.03	0.000000	0.000026	*Firmicutes*	*Clostridia*
DENOVO1030	3.43	-2.88	0.85	-3.39	0.000691	0.005825	*Firmicutes*	*Clostridia*
DENOVO418	4.86	-3.40	1.03	-3.29	0.001008	0.007476	*Firmicutes*	*Clostridia*
DENOVO255	21.59	-3.70	0.83	-4.44	0.000009	0.000261	*Firmicutes*	*Clostridia*
DENOVO892	4.33	-3.24	1.03	-3.13	0.001723	0.011125	*Firmicutes*	*Clostridia*
DENOVO162	24.93	-2.70	0.99	-2.73	0.006349	0.030592	*Firmicutes*	*Clostridia*
DENOVO243	8.50	-2.68	0.89	-3.01	0.002619	0.014950	*Firmicutes*	*Clostridia*
DENOVO290	13.43	-1.95	0.77	-2.53	0.011496	0.047388	*Firmicutes*	*Clostridia*
DENOVO234	26.20	-2.67	0.79	-3.37	0.000763	0.006154	*Firmicutes*	*Clostridia*
DENOVO335	8.68	-2.65	1.04	-2.54	0.011132	0.046941	*Firmicutes*	*Clostridia*

DENOVO161	18.26	-2.91	0.71	-4.12	0.000038	0.000765	*Firmicutes*	*Clostridia*
DENOVO254	7.29	-3.23	1.17	-2.77	0.005661	0.027637	*Firmicutes*	*Clostridia*
DENOVO98	10.59	-4.83	1.34	-3.61	0.000309	0.003372	*Firmicutes*	*Clostridia*
DENOVO550	12.87	-2.15	0.77	-2.79	0.005248	0.025962	*Firmicutes*	*Clostridia*
DENOVO259	7.30	-3.12	1.21	-2.59	0.009686	0.042276	*Firmicutes*	*Clostridia*
DENOVO239	17.91	-2.16	0.69	-3.13	0.001764	0.011125	*Firmicutes*	*Clostridia*
DENOVO115	32.37	-3.09	0.71	-4.36	0.000013	0.000343	*Firmicutes*	*Clostridia*
DENOVO647	5.30	-3.00	0.83	-3.63	0.000280	0.003188	*Firmicutes*	*Clostridia*
DENOVO141	32.38	-2.96	0.79	-3.73	0.000190	0.002514	*Firmicutes*	*Clostridia*
DENOVO308	26.95	1.88	0.66	2.84	0.004500	0.022871	*Firmicutes*	*Clostridia*
DENOVO218	148.32	6.20	1.04	5.98	0.000000	0.000000	*Firmicutes*	*Clostridia*
DENOVO439	6.17	-3.99	1.08	-3.68	0.000236	0.003000	*Firmicutes*	*Clostridia*
DENOVO176	29.28	-3.12	0.75	-4.15	0.000033	0.000719	*Firmicutes*	*Clostridia*
DENOVO90	25.29	-4.96	1.08	-4.59	0.000004	0.000138	*Firmicutes*	*Clostridia*
DENOVO1188	8.71	-3.37	0.89	-3.77	0.000166	0.002365	*Firmicutes*	*Clostridia*
DENOVO295	10.79	-2.90	0.83	-3.49	0.000478	0.004550	*Firmicutes*	*Clostridia*
DENOVO406	4.82	-2.28	0.74	-3.06	0.002237	0.013490	*Firmicutes*	*Clostridia*
DENOVO420	5.58	-2.52	0.97	-2.60	0.009361	0.041844	*Firmicutes*	*Clostridia*
DENOVO769	38.80	-4.05	1.03	-3.94	0.000080	0.001267	*Firmicutes*	*Clostridia*
DENOVO35	139.19	-2.56	0.81	-3.17	0.001516	0.010042	*Firmicutes*	*Clostridia*
DENOVO661	38.75	-2.63	0.87	-3.03	0.002449	0.014199	*Firmicutes*	*Clostridia*
DENOVO82	41.43	-2.19	0.81	-2.72	0.006554	0.031173	*Firmicutes*	*Clostridia*
DENOVO44	132.81	-2.49	0.66	-3.75	0.000179	0.002456	*Firmicutes*	*Clostridia*
DENOVO1494	25.40	-3.27	0.78	-4.19	0.000028	0.000688	*Firmicutes*	*Clostridia*
DENOVO490	16.71	-2.04	0.80	-2.54	0.011134	0.046941	*Firmicutes*	*Clostridia*
DENOVO177	16.71	-2.57	0.87	-2.96	0.003098	0.017154	*Firmicutes*	*Clostridia*
DENOVO1102	8.73	-3.01	1.07	-2.81	0.004912	0.024627	*Firmicutes*	*Clostridia*
DENOVO562	13.62	-2.76	0.75	-3.67	0.000243	0.003000	*Firmicutes*	*Clostridia*
DENOVO36	132.42	-2.26	0.72	-3.13	0.001769	0.011125	*Firmicutes*	*Clostridia*

DENOVO338	19.32	-2.81	0.86	-3.25	0.001168	0.008174	*Firmicutes*	*Clostridia*
DENOVO649	20.27	-3.19	0.78	-4.08	0.000044	0.000824	*Firmicutes*	*Clostridia*
DENOVO14	370.87	-2.30	0.58	-3.93	0.000085	0.001267	*Firmicutes*	*Clostridia*
DENOVO926	3.97	-2.97	0.86	-3.46	0.000534	0.004717	*Firmicutes*	*Clostridia*
DENOVO27	239.93	-1.64	0.63	-2.62	0.008725	0.039474	*Firmicutes*	*Clostridia*
DENOVO1190	6.53	-2.91	0.89	-3.27	0.001065	0.007598	*Firmicutes*	*Clostridia*
DENOVO322	9.32	-2.61	0.81	-3.21	0.001337	0.009188	*Firmicutes*	*Clostridia*
DENOVO457	11.29	-2.56	0.95	-2.70	0.006963	0.032698	*Firmicutes*	*Clostridia*
DENOVO264	7.65	-3.48	0.98	-3.56	0.000365	0.003758	*Firmicutes*	*Clostridia*
DENOVO6	106.19	3.79	0.76	4.98	0.000001	0.000028	*Firmicutes*	*Bacilli*
DENOVO13	14.62	3.99	0.83	4.79	0.000002	0.000057	*Firmicutes*	*Bacilli*
DENOVO2	73.14	1.60	0.44	3.66	0.000255	0.003047	*Firmicutes*	*Bacilli*
DENOVO207	85.07	2.81	0.71	3.94	0.000082	0.001267	*Proteobacteria*	*Deltaproteobacteria*
DENOVO187	29.31	2.06	0.70	2.93	0.003407	0.018121	*Proteobacteria*	*Deltaproteobacteria*
DENOVO480	17.97	4.94	0.94	5.23	0.000000	0.000016	*Proteobacteria*	*Deltaproteobacteria*
DENOVO383	21.48	3.29	0.94	3.52	0.000431	0.004212	*Proteobacteria*	*Deltaproteobacteria*
DENOVO189	3.21	4.82	1.33	3.63	0.000284	0.003188	*Proteobacteria*	*Gammaproteobacteria*
DENOVO245	15.31	6.87	1.34	5.12	0.000000	0.000023	*Proteobacteria*	*Betaproteobacteria*
DENOVO57	6.68	-1.67	0.58	-2.87	0.004127	0.021421	*Proteobacteria*	*Betaproteobacteria*
DENOVO642	4.76	3.18	1.07	2.97	0.003022	0.016989	*Proteobacteria*	*Betaproteobacteria*
DENOVO229	4.31	5.32	1.05	5.06	0.000000	0.000025	*Proteobacteria*	*Alphaproteobacteria*
DENOVO8	10728.81	5.60	0.77	7.30	0.000000	0.000000	*Deferribacteres*	*Deferribacteres*
DENOVO397	20.52	4.35	0.78	5.61	0.000000	0.000003	*Actinobacteria*	*Actinobacteria*
DENOVO309	28.20	2.71	0.68	4.01	0.000061	0.001082	*Bacteroidetes*	*Bacteroidia*
DENOVO230	8.06	-2.11	0.66	-3.19	0.001416	0.009550	*Bacteroidetes*	*Bacteroidia*
DENOVO22	81.87	-2.53	0.77	-3.28	0.001045	0.007598	*Bacteroidetes*	*Bacteroidia*
DENOVO219	6.05	-2.74	0.76	-3.59	0.000326	0.003460	*Bacteroidetes*	*Bacteroidia*
DENOVO48	53.62	-2.70	0.77	-3.53	0.000410	0.004115	*Bacteroidetes*	*Bacteroidia*
DENOVO74	23.80	-2.14	0.70	-3.05	0.002254	0.013490	*Bacteroidetes*	*Bacteroidia*

DENOVO104	17.42	-2.46	0.95	-2.59	0.009615	0.042276	*Bacteroidetes*	*Bacteroidia*
DENOVO357	1.94	-2.07	0.72	-2.87	0.004157	0.021421	*Bacteroidetes*	*Bacteroidia*
DENOVO66	45.23	-2.36	0.71	-3.30	0.000963	0.007295	*Bacteroidetes*	*Bacteroidia*
DENOVO146	10.62	-2.24	0.67	-3.36	0.000787	0.006211	*Bacteroidetes*	*Bacteroidia*
DENOVO150	17.04	-3.37	0.81	-4.18	0.000030	0.000688	*Bacteroidetes*	*Bacteroidia*
DENOVO96	20.00	-1.89	0.54	-3.48	0.000504	0.004678	*Bacteroidetes*	*Bacteroidia*
DENOVO165	13.00	-2.50	0.82	-3.06	0.002196	0.013490	*Bacteroidetes*	*Bacteroidia*
DENOVO279	8.06	-2.05	0.81	-2.53	0.011485	0.047388	*Bacteroidetes*	*Bacteroidia*
DENOVO467	18.89	-2.14	0.73	-2.93	0.003419	0.018121	*Bacteroidetes*	*Bacteroidia*
DENOVO81	17.71	-2.42	0.72	-3.37	0.000740	0.006101	*Bacteroidetes*	*Bacteroidia*
DENOVO30	56.53	-3.26	0.83	-3.95	0.000077	0.001267	*Bacteroidetes*	*Bacteroidia*
DENOVO93	23.14	-2.94	0.87	-3.40	0.000683	0.005825	*Bacteroidetes*	*Bacteroidia*
DENOVO46	75.31	-1.89	0.46	-4.11	0.000039	0.000765	*Bacteroidetes*	*Bacteroidia*
DENOVO19	84.65	-2.83	0.57	-4.97	0.000001	0.000028	*Bacteroidetes*	*Bacteroidia*

Table A.6.9: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the caecum and *Trichuris muris*.

OTU	Base Mean	$\log^2$ fold change	$\log^2$ fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO159	19.15	-5.10	1.76	-2.89	0.003861	0.020457	*Firmicutes*	*Clostridia*
DENOVO283	90.79	-5.47	2.01	-2.73	0.006354	0.029262	*Firmicutes*	*Clostridia*
DENOVO533	147.68	-3.63	1.23	-2.95	0.003202	0.018925	*Firmicutes*	*Clostridia*
DENOVO211	44.21	-2.93	1.15	-2.55	0.010805	0.041166	*Firmicutes*	*Clostridia*
DENOVO116	52.42	-3.15	1.29	-2.44	0.014619	0.048836	*Firmicutes*	*Clostridia*
DENOVO336	46.24	-4.31	1.50	-2.87	0.004127	0.021327	*Firmicutes*	*Clostridia*
DENOVO363	15.68	-5.23	1.37	-3.81	0.000139	0.002155	*Firmicutes*	*Clostridia*

DENOVO140	76.64	-2.55	1.03	-2.48	0.013126	0.045630	*Firmicutes*	*Clostridia*
DENOVO263	26.95	-3.67	1.07	-3.42	0.000636	0.006235	*Firmicutes*	*Clostridia*
DENOVO423	12.96	-5.25	1.34	-3.93	0.000085	0.001574	*Firmicutes*	*Clostridia*
DENOVO538	2.80	-4.25	1.55	-2.73	0.006261	0.029155	*Firmicutes*	*Clostridia*
DENOVO680	6.96	-4.88	1.74	-2.81	0.004994	0.024337	*Firmicutes*	*Clostridia*
DENOVO702	14.90	-4.65	1.40	-3.33	0.000870	0.007571	*Firmicutes*	*Clostridia*
DENOVO68	508.35	-2.88	1.01	-2.87	0.004164	0.021327	*Firmicutes*	*Clostridia*
DENOVO288	28.47	-3.92	1.52	-2.57	0.010119	0.038981	*Firmicutes*	*Clostridia*
DENOVO516	7.47	-4.35	1.76	-2.48	0.013138	0.045630	*Firmicutes*	*Clostridia*
DENOVO524	7.09	-5.18	1.78	-2.91	0.003618	0.019663	*Firmicutes*	*Clostridia*
DENOVO329	27.46	-3.99	1.34	-2.99	0.002791	0.017580	*Firmicutes*	*Clostridia*
DENOVO479	14.72	-5.87	1.29	-4.54	0.000006	0.000163	*Firmicutes*	*Clostridia*
DENOVO733	6.80	-5.05	1.96	-2.57	0.010147	0.038981	*Firmicutes*	*Clostridia*
DENOVO137	165.91	-3.10	1.16	-2.67	0.007506	0.031745	*Firmicutes*	*Clostridia*
DENOVO33	210.39	-2.70	1.09	-2.47	0.013574	0.046353	*Firmicutes*	*Clostridia*
DENOVO234	108.01	-3.19	1.20	-2.66	0.007849	0.032681	*Firmicutes*	*Clostridia*
DENOVO898	17.75	-5.54	2.15	-2.58	0.009893	0.038979	*Firmicutes*	*Clostridia*
DENOVO152	206.10	-4.61	1.38	-3.33	0.000862	0.007571	*Firmicutes*	*Clostridia*
DENOVO161	70.13	-3.59	1.11	-3.23	0.001234	0.009482	*Firmicutes*	*Clostridia*
DENOVO67	52.87	-4.22	1.60	-2.64	0.008321	0.033648	*Firmicutes*	*Clostridia*
DENOVO652	5.42	-4.05	1.60	-2.54	0.011063	0.041802	*Firmicutes*	*Clostridia*
DENOVO125	47.30	-3.67	1.47	-2.49	0.012617	0.045630	*Firmicutes*	*Clostridia*
DENOVO87	163.73	-2.87	0.98	-2.91	0.003569	0.019663	*Firmicutes*	*Clostridia*
DENOVO468	10.60	-5.55	1.97	-2.82	0.004854	0.024337	*Firmicutes*	*Clostridia*
DENOVO284	33.70	-2.60	1.05	-2.49	0.012939	0.045630	*Firmicutes*	*Clostridia*
DENOVO239	91.92	-3.21	1.15	-2.79	0.005340	0.025379	*Firmicutes*	*Clostridia*
DENOVO115	164.36	-2.84	1.06	-2.69	0.007123	0.030687	*Firmicutes*	*Clostridia*
DENOVO995	13.59	-5.01	1.57	-3.20	0.001384	0.010130	*Firmicutes*	*Clostridia*
DENOVO141	164.88	-3.21	1.09	-2.94	0.003293	0.019217	*Firmicutes*	*Clostridia*

DENOVO256	35.21	-4.59	1.31	-3.49	0.000474	0.004970	*Firmicutes*	*Clostridia*
DENOVO439	20.85	-7.01	1.66	-4.22	0.000024	0.000560	*Firmicutes*	*Clostridia*
DENOVO355	33.47	-6.91	1.26	-5.47	0.000000	0.000003	*Firmicutes*	*Clostridia*
DENOVO820	5.19	-4.84	1.94	-2.49	0.012725	0.045630	*Firmicutes*	*Clostridia*
DENOVO365	10.97	-5.57	1.66	-3.36	0.000791	0.007294	*Firmicutes*	*Clostridia*
DENOVO176	163.51	-3.75	1.13	-3.31	0.000917	0.007774	*Firmicutes*	*Clostridia*
DENOVO608	2.53	-3.06	1.17	-2.61	0.008981	0.036002	*Firmicutes*	*Clostridia*
DENOVO311	47.95	-3.68	1.39	-2.66	0.007869	0.032681	*Firmicutes*	*Clostridia*
DENOVO571	13.00	-6.04	1.76	-3.43	0.000610	0.006118	*Firmicutes*	*Clostridia*
DENOVO1183	4.03	-4.18	1.69	-2.47	0.013344	0.045907	*Firmicutes*	*Clostridia*
DENOVO177	76.55	-3.76	1.21	-3.10	0.001934	0.013310	*Firmicutes*	*Clostridia*
DENOVO1102	15.75	-3.93	1.40	-2.80	0.005068	0.024337	*Firmicutes*	*Clostridia*
DENOVO876	3.07	-3.28	1.32	-2.48	0.013164	0.045630	*Firmicutes*	*Clostridia*
DENOVO1326	4.34	-4.47	1.53	-2.91	0.003565	0.019663	*Firmicutes*	*Clostridia*
DENOVO1622	2.69	-4.15	1.51	-2.74	0.006098	0.028686	*Firmicutes*	*Clostridia*
DENOVO599	13.93	-3.82	1.40	-2.72	0.006479	0.029262	*Firmicutes*	*Clostridia*
DENOVO884	23.46	-4.16	1.10	-3.79	0.000153	0.002202	*Firmicutes*	*Clostridia*
DENOVO1417	3.20	-4.42	1.72	-2.57	0.010111	0.038981	*Firmicutes*	*Clostridia*
DENOVO27	1164.89	-3.03	1.02	-2.98	0.002860	0.017580	*Firmicutes*	*Clostridia*
DENOVO1330	5.98	-4.28	1.47	-2.91	0.003668	0.019663	*Firmicutes*	*Clostridia*
DENOVO535	20.75	-5.88	1.66	-3.55	0.000386	0.004235	*Firmicutes*	*Clostridia*
DENOVO1190	16.74	-3.88	1.19	-3.25	0.001142	0.008968	*Firmicutes*	*Clostridia*
DENOVO658	3.11	-4.62	1.36	-3.40	0.000686	0.006457	*Firmicutes*	*Clostridia*
DENOVO60	2.80	-3.44	1.18	-2.92	0.003551	0.019663	*Firmicutes*	*Bacilli*
DENOVO13	8.50	-5.55	1.75	-3.18	0.001466	0.010558	*Firmicutes*	*Bacilli*
DENOVO615	5.18	-5.53	1.42	-3.89	0.000101	0.001787	*Proteobacteria*	*Deltaproteobacteria*
DENOVO187	24.89	-6.15	1.33	-4.63	0.000004	0.000123	*Proteobacteria*	*Deltaproteobacteria*
DENOVO383	3.86	-4.87	1.65	-2.95	0.003179	0.018925	*Proteobacteria*	*Deltaproteobacteria*
DENOVO190	17.23	-7.56	1.23	-6.16	0.000000	0.000000	*Proteobacteria*	*Deltaproteobacteria*

DENOVO189	3.57	4.60	1.81	2.53	0.011321	0.041872	*Proteobacteria*	*Gammaproteobacteria*
DENOVO29	8.99	-4.50	1.70	-2.64	0.008237	0.033606	*Proteobacteria*	*Gammaproteobacteria*
DENOVO386	18.52	-3.67	1.23	-2.98	0.002917	0.017694	*Proteobacteria*	*Betaproteobacteria*
DENOVO57	28.10	-5.68	1.11	-5.14	0.000000	0.000011	*Proteobacteria*	*Betaproteobacteria*
DENOVO394	3.18	4.56	1.82	2.51	0.012115	0.044325	*Proteobacteria*	*Alphaproteobacteria*
DENOVO180	5.17	4.30	1.04	4.13	0.000036	0.000797	*Proteobacteria*	*Alphaproteobacteria*
DENOVO8	736.13	-3.17	1.18	-2.68	0.007257	0.030976	*Deferribacteres*	*Deferribacteres*
DENOVO241	31.96	-3.34	1.01	-3.29	0.000992	0.008023	*Actinobacteria*	*Actinobacteria*
DENOVO436	5.65	-4.10	1.31	-3.12	0.001778	0.012420	*Actinobacteria*	*Actinobacteria*
DENOVO553	2.36	-3.90	1.36	-2.87	0.004044	0.021188	*Actinobacteria*	*Actinobacteria*
DENOVO397	3.85	-5.10	1.36	-3.76	0.000170	0.002375	*Actinobacteria*	*Actinobacteria*
DENOVO421	5.64	-5.37	1.27	-4.24	0.000022	0.000541	*Actinobacteria*	*Actinobacteria*
DENOVO433	5.49	-5.30	1.73	-3.06	0.002185	0.014188	*Actinobacteria*	*Actinobacteria*
DENOVO294	17.25	-7.36	1.32	-5.58	0.000000	0.000002	*Actinobacteria*	*Actinobacteria*
DENOVO465	47.31	-4.24	1.27	-3.34	0.000848	0.007571	*Bacteroidetes*	*Bacteroidia*
DENOVO235	49.64	-3.80	1.43	-2.65	0.007975	0.032825	*Bacteroidetes*	*Bacteroidia*
DENOVO428	13.72	-5.24	1.40	-3.74	0.000185	0.002431	*Bacteroidetes*	*Bacteroidia*
DENOVO24	335.71	-4.09	1.52	-2.69	0.007110	0.030687	*Bacteroidetes*	*Bacteroidia*
DENOVO40	263.88	-3.59	1.32	-2.72	0.006538	0.029262	*Bacteroidetes*	*Bacteroidia*
DENOVO309	20.79	-3.21	1.27	-2.53	0.011354	0.041872	*Bacteroidetes*	*Bacteroidia*
DENOVO1618	3.61	-5.14	1.45	-3.54	0.000403	0.004316	*Bacteroidetes*	*Bacteroidia*
DENOVO17	363.21	-3.44	1.04	-3.30	0.000952	0.007836	*Bacteroidetes*	*Bacteroidia*
DENOVO89	34.49	-7.26	1.33	-5.46	0.000000	0.000003	*Bacteroidetes*	*Bacteroidia*
DENOVO230	46.96	-4.37	1.32	-3.31	0.000927	0.007774	*Bacteroidetes*	*Bacteroidia*
DENOVO84	84.33	-3.59	1.47	-2.45	0.014451	0.048836	*Bacteroidetes*	*Bacteroidia*
DENOVO129	133.46	-7.87	1.52	-5.18	0.000000	0.000010	*Bacteroidetes*	*Bacteroidia*
DENOVO541	4.05	-5.31	1.46	-3.63	0.000283	0.003428	*Bacteroidetes*	*Bacteroidia*
DENOVO48	195.70	-3.49	1.12	-3.13	0.001762	0.012420	*Bacteroidetes*	*Bacteroidia*
DENOVO74	143.87	-4.23	1.18	-3.59	0.000331	0.003914	*Bacteroidetes*	*Bacteroidia*

DENOVO138	44.46	-3.64	1.34	-2.71	0.006682	0.029526	*Bacteroidetes*	*Bacteroidia*
DENOVO104	46.87	-4.50	1.26	-3.58	0.000342	0.003947	*Bacteroidetes*	*Bacteroidia*
DENOVO357	8.01	-4.14	1.09	-3.80	0.000145	0.002155	*Bacteroidetes*	*Bacteroidia*
DENOVO472	6.33	-5.15	1.35	-3.80	0.000143	0.002155	*Bacteroidetes*	*Bacteroidia*
DENOVO66	180.23	-2.87	1.18	-2.44	0.014562	0.048836	*Bacteroidetes*	*Bacteroidia*
DENOVO252	38.72	-6.40	1.40	-4.56	0.000005	0.000155	*Bacteroidetes*	*Bacteroidia*
DENOVO126	101.29	-6.61	1.92	-3.44	0.000573	0.005870	*Bacteroidetes*	*Bacteroidia*
DENOVO117	72.54	-6.79	1.45	-4.69	0.000003	0.000103	*Bacteroidetes*	*Bacteroidia*
DENOVO150	44.03	-5.02	1.27	-3.95	0.000079	0.001545	*Bacteroidetes*	*Bacteroidia*
DENOVO31	134.27	-3.77	1.06	-3.55	0.000384	0.004235	*Bacteroidetes*	*Bacteroidia*
DENOVO96	49.16	-2.96	0.92	-3.22	0.001285	0.009552	*Bacteroidetes*	*Bacteroidia*
DENOVO391	20.31	-6.52	1.40	-4.67	0.000003	0.000107	*Bacteroidetes*	*Bacteroidia*
DENOVO58	146.87	-3.37	1.20	-2.80	0.005058	0.024337	*Bacteroidetes*	*Bacteroidia*
DENOVO251	11.97	-5.21	1.70	-3.07	0.002145	0.014129	*Bacteroidetes*	*Bacteroidia*
DENOVO269	48.57	-4.22	1.13	-3.75	0.000180	0.002431	*Bacteroidetes*	*Bacteroidia*
DENOVO526	1.87	-4.16	1.64	-2.53	0.011333	0.041872	*Bacteroidetes*	*Bacteroidia*
DENOVO486	5.17	-5.26	1.70	-3.09	0.001993	0.013513	*Bacteroidetes*	*Bacteroidia*
DENOVO279	38.89	-3.53	1.30	-2.71	0.006725	0.029526	*Bacteroidetes*	*Bacteroidia*
DENOVO101	125.87	-6.54	1.08	-6.04	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO83	123.03	-6.10	1.60	-3.82	0.000135	0.002155	*Bacteroidetes*	*Bacteroidia*
DENOVO47	131.61	-5.00	1.16	-4.31	0.000017	0.000439	*Bacteroidetes*	*Bacteroidia*
DENOVO467	69.75	-3.63	1.12	-3.25	0.001148	0.008968	*Bacteroidetes*	*Bacteroidia*
DENOVO81	54.59	-3.45	1.18	-2.92	0.003466	0.019663	*Bacteroidetes*	*Bacteroidia*
DENOVO242	36.32	-3.94	1.51	-2.60	0.009361	0.037202	*Bacteroidetes*	*Bacteroidia*
DENOVO134	65.35	-5.84	1.05	-5.58	0.000000	0.000002	*Bacteroidetes*	*Bacteroidia*
DENOVO56	101.82	-3.05	1.02	-2.98	0.002853	0.017580	*Bacteroidetes*	*Bacteroidia*
DENOVO286	19.72	-7.71	1.22	-6.33	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO30	371.50	-4.01	1.24	-3.22	0.001277	0.009552	*Bacteroidetes*	*Bacteroidia*
DENOVO114	109.13	-4.91	1.25	-3.94	0.000080	0.001545	*Bacteroidetes*	*Bacteroidia*

DENOVO225	38.08	-6.71	1.18	-5.70	0.000000	0.000001	*Bacteroidetes*	*Bacteroidia*
DENOVO109	106.55	-4.29	1.12	-3.85	0.000120	0.002051	*Bacteroidetes*	*Bacteroidia*
DENOVO93	98.16	-5.27	1.42	-3.71	0.000208	0.002587	*Bacteroidetes*	*Bacteroidia*
DENOVO73	103.00	-3.76	1.25	-3.02	0.002552	0.016343	*Bacteroidetes*	*Bacteroidia*
DENOVO46	295.33	-3.60	1.06	-3.40	0.000665	0.006386	*Bacteroidetes*	*Bacteroidia*
DENOVO158	44.33	-4.36	1.50	-2.91	0.003639	0.019663	*Bacteroidetes*	*Bacteroidia*
DENOVO19	551.77	-4.97	1.16	-4.30	0.000017	0.000439	*Bacteroidetes*	*Bacteroidia*
DENOVO11	789.16	-3.19	1.17	-2.72	0.006479	0.029262	*Bacteroidetes*	*Bacteroidia*
DENOVO45	235.14	-3.67	1.19	-3.08	0.002084	0.013925	*Bacteroidetes*	*Bacteroidia*
DENOVO120	57.21	-7.63	1.04	-7.37	0.000000	0.000000	*Bacteroidetes*	*Bacteroidia*
DENOVO108	180.79	-3.51	1.41	-2.49	0.012926	0.045630	*Bacteroidetes*	*Bacteroidia*
DENOVO39	676.34	-4.94	1.23	-4.01	0.000062	0.001297	*Bacteroidetes*	*Bacteroidia*
DENOVO34	305.54	-5.40	1.45	-3.72	0.000197	0.002527	*Bacteroidetes*	*Bacteroidia*
DENOVO4	7.25	-4.81	1.71	-2.81	0.004955	0.024337	*Tenericutes*	*Mollicutes*
DENOVO61	222.64	-2.95	1.05	-2.81	0.004901	0.024337	*Candidatus Saccharibacteria*	*Saccharibacteria*

Table A.6.10: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the proximal colon and *Syphacia frederici.*

OTU	Base Mean	Log^2 fold change	lfcSE	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
DENOVO38	6390.51	8.56	1.89	4.54	0.000006	0.002366	*Proteobacteria*	*Gammaproteobacteria*
DENOVO397	11.31	5.71	1.12	5.11	0.000000	0.000262	*Actinobacteria*	*Actinobacteria*

Table A.6.11: Output table of statistics from DESeq analyses used to identify significant changes in OTU abundances (p <0.05) and their respective fold changes ($\log^2$) between the distal colon and Mastophorus *muris.*

OTU	Base Mean	Log^2 fold	Log^2 fold	DESeq	*p*-value	Adjusted	Phylum	Class

		change	**change standard error**	**statistic**		***p*-value**		
DENOVO1	12307.24	11.08	1.97	5.63	0.000000	0.000012	*Proteobacteria*	*Gammaproteobacteria*

Appendix A.7: Supplementary table of data presented in Chapter 6

Table A.7.1: Sampling regime of wild mice (*Apodemus flavicollis*) faecal samples used for a helminth egg transplant experiment. Faeces were used as either an egg donor and/or recipient of *Heligmosomoides polygyrus* and *Trichuris muris* eggs. Due to variation in faecal yield and egg counts not all individuals were used as both a donor and a recipient. In addition, when faeces were adequate faecal egg count and microbiota analyses were performed for some samples.

Mouse ID	*H. polygyrus*		*T. muris*		Faecal egg count analysis	Microbiota analysis
	Egg donor	Egg recipient	Egg donor	Egg recipient		
Mouse 1	✓	✓	✓	✓	✓	✓
Mouse 2	✓	✓	✗	✓	✓	✓
Mouse 3	✗	✓	✗	✓	✓	✓
Mouse 4	✓	✓	✓	✓	✓	✓
Mouse 5	✓	✓	✗	✓	✓	✓
Mouse 6	✓	✓	✗	✗	✓	✓
Mouse 7	✗	✓	✗	✗	✓	✓
Mouse 8	✓	✓	✓	✓	✓	✓
Mouse 9	✓	✓	✗	✗	✓	✗
Mouse 10	✗	✓	✗	✗	✓	✗
Mouse 11	✗	✗	✗	✓	✓	✗
Mouse 12	✗	✗	✓	✓	✓	✗
Mouse 13	✗	✗	✓	✓	✗	✗
Mouse 14	✗	✗	✗	✓	✗	✗
TOTAL	**7**	**10**	**5**	**10**	**12**	**8**

Table A.7.2: Break down of recipient-donor combinations of *Apodemus flavicollis* individuals used in a transplant experiment of *Heligmosomoides polygyrus* eggs. The number of culture replicates for each combination across the course of the experiment is presented.

Recipient ID	Donor ID	Culture type	No. of replicates
Mouse 1	Mouse 1	Self	2
Mouse 2	Mouse 9	Non-self	5

Mouse 2	Mouse 2	Self	1
Mouse 3	Mouse 6	Non-self	2
Mouse 3	Mouse 4	Non-self	1
Mouse 3	Mouse 2	Non-self	1
Mouse 4	Mouse 4	Self	2
Mouse 5	Mouse 5	Self	1
Mouse 5	Mouse 8	Non-self	2
Mouse 9	Mouse 9	Self	3
Mouse 6	Mouse 1	Non-self	1
Mouse 6	Mouse 6	Self	3
Mouse 6	Mouse 8	Non-self	2
Mouse 10	Mouse 4	Non-self	1
Mouse 7	Mouse 6	Non-self	1
Mouse 8	Mouse 8	Self	4

Table A.7.3: Break down of recipient-donor combinations of Apodemus flavicollis individuals used in a transplant experiment of Trichuris muris eggs. The number of culture replicates for each combination across the course of the experiment is presented.

Recipient ID	Donor ID	Culture type	No. of replicates
Mouse 1	Mouse 1	Self	4
Mouse 11	Mouse 12	Non-self	1
Mouse 12	Mouse 12	Self	1
Mouse 13	Mouse 1	Non-self	2
Mouse 13	Mouse 13	Self	1
Mouse 13	Mouse 8	Non-self	1
Mouse 13	Mouse 1	Non-self	1
Mouse 14	Mouse 4	Non-self	1
Mouse 2	Mouse 4	Non-self	1
Mouse 3	Mouse 1	Non-self	2
Mouse 4	Mouse 4	Self	3
Mouse 5	Mouse 8	Non-self	2
Mouse 8	Mouse 8	Self	3
Mouse 8	Mouse 13	Non-self	1

Mouse 8	Mouse 1	Non-self	1

Appendix A.8: Supplementary thesis information

Papers that have been published during the course of the Ph. D., but which have not directly contributed to the thesis.

Bibliography

A.8.1

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A.8.3

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