An Ecological Approach to Understanding Gut Microbiota and Macrobiota Interactions

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Frontispiece

Emily Pascoe,
Goes where the mice go,
Goes where the voles go,
But mostly near Trento.

Parasites! Parasites!
Up their bums and in their poo.
Parasites! Parasites!
There are microbes in there too.

Emily Pascoe,
Fiat Panda goes so slow,
The headlights have no glow,
No handbrake, but hey ho?!

Parasites! Parasites!
Looking in their guts to see.
Parasites! Parasites!
What happens when they're helminth free?

Beware the foxes and the stoats,
They didn't come for the oats...
They came for the mice!
They came in the night(sss)!!!
10 inch nails couldn't keep them away,
There was only one person who could save the day!

And her name is...
Emily Pascoe, Emily Pascoe
EMILY PASCOE!!!

"*Apodemus flavicollis*" by Nia Thomas

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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

Despite a plethora of research on the positive and negative impacts of gut microbiota (community of micro-organisms) and macrobiota (parasitic helminths), as yet there is little focus on how these two sympatric and ubiquitous communities interact. Given that there are increasing evolutionary pressures imposed on microbiota and macrobiota, which have currently unknown system-wide implications, e.g., antibiotic and anthelmintic treatment, it is timely to investigate microbiota-macrobiota interactions. This thesis uses an ecological approach to understand microbiota-macrobiota interactions in a wild rodent system. First, a review of animal gut microbiota literature established the current research landscape of this topic, which highlighted the lack of studies on wild animals, despite the advantages that these animals can provide, e.g., as model systems (Chapter 2). In addition, perturbation field experiments were used to tease apart microbiota-macrobiota interactions in a wild rodent. The impact of helminth removal (using anthelmintic) on microbiota was investigated, which revealed that, with the exception of faecal microbiota composition, gut bacterial communities remained stable following anthelmintic treatment (Chapter 3). Following perturbation of the microbiota (using antibiotic), both fecundity and size of helminths increased (Chapter 4). Helminths were found to be associated with a microbiota that exhibits interspecific variation as well as intraspecific variation, which was driven by gut location of helminths, although 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 in host faeces, compared to faeces from another individual, indicating that faecal microbiota may have some resistance to helminth development (Chapter 6). This thesis highlights the importance of considering systemwide implications of a treatment or perturbation, particularly on gut microbiota-macrobiota interactions.

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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, the current knowledge on gut microbiota and macrobiota are briefly reviewed, followed by a synopsis of the literature on microbiota-macrobiota interactions, which are given more attention in each of the relevant data chapters. The study system used in this thesis is a wild rodent, namely the yellow-necked mouse (*Apodemus flavicollis*) in northeastern Italy. 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 describe the taxonomic composition of a microbial community (Marchesi and Ravel, 2015). Every niche of an organism is inhabited by microbes, 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 above-ground phyllosphere of plants (Lindow and Brandl, 2003). The microbial communities inhabiting each niche have a highly specific composition, for example, microbiota composition varies between each tooth of an individual (Bik *et al.*, 2010), and differs between the crypts and the lumen of the colon (Pédron *et al.*, 2012). The number of microbial cells associated with a host often exceeds the number of autochthonous cells; for example, in mammals microbes are estimated to outnumber host cells by around ten times (Palmer *et al.*, 2007).

In vertebrates, the gut harbours the most densely populated and diverse microbiota of the body. Humans typically possess 10^{11}-10^{12} microbes/ml of luminal content (Palmer *et al.*, 2007), comprised of an estimated 500 to 1,000 species (Hrncir *et al.*, 2008), which equates to a genome consisting of 150 times more genes than that of a human (Gill *et al.*, 2006). Gut microbiota composition continually changes throughout the lifespan of an individual (Lozupone *et al.*, 2012; Rodríguez *et al.*, 2015). 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 (Jiménez *et al.*, 2008). However, the first critical inoculum that has significant impacts on the host is received during birth (Dominguez-Bello *et al.*, 2010; Jakobsson *et al.*, 2014), when the gut of vaginally delivered babies is initially colonised by maternal gut (faecal) and vaginal microbes (Dominguez-Bello *et al.*, 2010; Jakobsson *et al.*, 2014). However, the guts of individuals delivered by caesarean section are instead colonised by microbes typically found on the skin; this difference in birth inoculum significantly affects maturation of the immune system (Dominguez-Bello *et al.*, 2010; Jakobsson *et al.*, 2014; Figure 1.1). Consequently, caesarean section born individuals are more likely to be susceptible to autoimmune diseases (Dominguez-Bello *et al.*, 2010; Jakobsson *et al.*, 2014). Hence, in humans the mode of birth can have lifelong consequences.

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 comparatively stable, but can still fluctuate (Koenig *et al.*, 2011; Faith *et al.*, 2013). A study on adult humans found that 40% of bacterial OTUs previously identified were no longer present in the gut when analyses

were repeated five years later (Faith *et al.*, 2013). 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), and environmental characteristics such as diet (Gibson *et al.*, 2004), and seasonality (Carey *et al.*, 2013; Jia *et al.*, 2013). Therefore, as a result of experience and exposure, the microbiota can vary greatly between individuals within the same species, and within an individual throughout time (e.g., Benson *et al.*, 2010; Faith *et al.*, 2013; Rodríguez *et al.*, 2015). 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 be categorised into disparate groups, as variation between individuals exists along a gradient (Jeffery *et al.*, 2012).

Microbiota studies have propelled, but have also been driven by, advances in technologies that characterise microbiota composition and functions, such as multi-'omic platforms including metataxonomics and metagenomics (Marchesi and Ravel, 2015). The subsequent plethora of gut microbiota studies have been motivated by knowledge that this community is vital for host health and physiological processes, thus research on this 'microbial organ' (Bäckhed *et al.*, 2005) has rapidly expanded, and continues to do so (Marchesi and Ravel, 2015). The relationship between the host and its microbiota is largely mutualistic: in return for nutrients from gut contents, the microbiota is essential to the host for chemical functions within the body, including 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'). Dysbiosis has been associated with 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 micro-organisms that are pathogenic in the gut include some strains of *Escherichia coli*, while other micro-organisms, such as *Clostridium difficile*, are usually benign, but can become pathogenic under certain dysbiotic conditions, such as when there is a deficiency in the bacteria which normally suppress over-growth, allowing *C. difficile* to proliferate (Aas *et al.*, 2003).

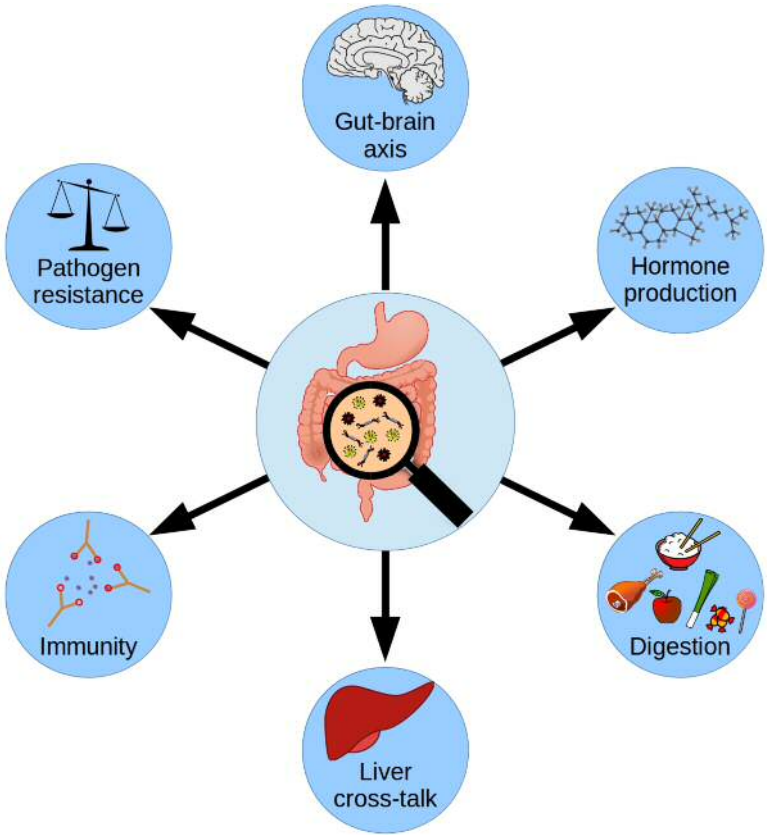

Figure 1.1: Gut microbiota studies have been driven by the knowledge that microbes are involved in many crucial functions within the host, including resistance to pathogens, immune system development and functions, digestion and hormone production, as well as interactions with organs such as the brain and liver.

Microbiota also plays a role in cognition, emotion and behaviour exhibited by the host (Figure 1.1). The gut-brain axis describes the bidirectional interactions that occur between the microbiota

and the central nervous system, which result from 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 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). In the laboratory, behaviours associated with anxiety are reduced in both germ-free mice (Diaz Heijtz *et al.*, 2011), and in mice administered the probiotic *Lactobacillus rhamnosus* (see Bravo *et al.*, 2011). Moreover, 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). The microbiota is associated with another vital organ; the liver, 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).

Microbiota also plays a crucial role in immune system functions in the host. A layer of just 30 µm of intestinal epithelial cells separates potential pathogens ingested by the host from the other internal organs of the body, as well as the circulatory, respiratory and other systems (Cahenzli *et al.*, 2012), thus microbiota composition 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 the immune cells and immune system: gut microbes promote lymphocyte and immunoglobulin production (Round and Mazmanian, 2009; Cahenzli *et al.*, 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 affect the ability of bacteria to colonise the gut (Rolfe *et al.*, 1981). Indeed, 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

As a result of the impact that gut microbiota has on host health (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 microbiota can be manipulated or modulated to incite health benefits and treat disease. Antibiotics, which were discovered in the early 1900s, have been widely administered to kill or prevent the proliferation of pathogenic bacteria since the 1940s (Aminov, 2010; Hauser, 2012). However, antibiotics usually function on a 'broad-spectrum', and induce changes in the entire microbial composition, by also affecting non-target and non-pathogenic bacteria, which can exacerbate or even cause dysbiosis (Francino, 2016). Impacts on microbiota that result from antibiotic treatment can be long-term; in humans antibiotic associated perturbation of gut microbiota is significant 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; for example, hundreds of tonnes are used annually in salmon farms in Chile alone (Cabello *et al.*, 2013). In addition, it is currently unknown if antibiotics also affect other components of the gut biome (including viruses, protozoa and macroparasites). Instead, treatments which promote the natural community of

microbes, such as probiotics and prebiotics, may be used to treat dysbiosis, and incite other benefits to the host 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 ingested both intentionally and unintentionally by humans and wild animals, and are administered to livestock to directly improve gut microbiota composition. Probiotics have been consumed by humans for centuries in fermented foods such as dairy products and preserved meats, albeit without specific intention (Soomro *et al.*, 2002). As knowledge on beneficial microbes has grown, testing and subsequent production of 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). As part of their normal behavioural repertoire, wildlife, such as the Kudzu bug (*Megacopta cribraria*) and bumble bees (*Bombus terrestris*), may consume probiotics, for example in the form of maternal symbiotic capsules, which prevent disease and improve general gut health (Hosokawa *et al.*, 2008; Koch and Schmid-Hempel, 2011).

Although commercial 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, or ingesting tablets or capsules containing bacterial communities derived from faecal microbiota, from a healthy individual into the gut of a recipient suffering severe dysbiosis, whereby faecal bacteria act as a multi-species probiotic for the

recipient (Lagier, 2014). FMT has proved successful in relieving symptoms of otherwise difficult to treat infections such as *C. difficile* (e.g., 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 a lack of studies on long-term impacts and potential risks associated with transferring an entire faecal microbiota between individuals. For example, infectious pathogens from the faecal donor may also be transferred to the recipient. In addition, 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 category 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). 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, which interfere with pathogen functions (Coutinho-Abreu *et al.*, 2010). Paratransgenesis may have a role in biocontrol for disease vectors, as the host is also less competent at vectoring pathogens after paratransgenesis administration, and transmission of the symbiont throughout an animal population is 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, such as carbohydrates, 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 acts as an arguably less refined prebiotic, and both diet composition and quantity can have major impacts on microbiota, which are both rapid and reproducible (Desai *et al.*, 2012; Deusch *et al.*, 2014; Roggenbuck *et al.*, 2014; Sonnenburg and Bäckhed, 2016), thus can be a powerful tool for modulating microbiota.

1.4 Sharing the gut: parasitic helminths – the macrobiota

The gut not only hosts the microbiota, but harbours an interacting biome of multiple organisms, including macroparasites (multicellular parasites). Although not as ubiquitous as the microbiota, macroparasite infections are the norm: 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). Although helminths can infect the majority of organs in the body, including 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 'macrobiota'.

Low level helminth infections can be relatively benign and well tolerated by the host, but high intensity helminth infections can have sub-lethal effects on the host, such as malnutrition, appetite loss, anaemia and reduced fecundity, and are consequently considered one of the main causes of poor productivity and ill health in domesticated animals (Beaver, 1975; Shetty, 2010; Sutherland and Scott, 2010). Despite these negative effects, it is important to note that an absence or reduction in helminth infections, as observed in most westernised societies (where there is better access to healthcare and flushing toilets, breaking the life-cycle of faecal-oral transmitted species), is not necessarily positive for host health (Bilbo *et al.*, 2011). A rise in the prevalence of auto- and hyperimmune diseases 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 immunoglobulin antibodies is similar to that observed during autoimmunity; however, the physiological response differs: the immune regulatory network is strengthened by a consequential response by T-helper 2 (T_h2) 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), or ingesting an infected intermediate host, such as for *Hymenolepis* species (see Baker, 2008). Other macroparasites such as *Ancylostoma duodenale* infect the host by penetrating the skin (Bethony *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 stages are necessarily parasitic, and may occur in the environment (Figure 1.2). For example, *Trichuris*

suis are infective from the first larval stage (L1) after eggs have hatched in the environment, however, *Heligmosomoides polygyrus* hatch in the environment but are 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* (see Bethony *et al.*, 2006).

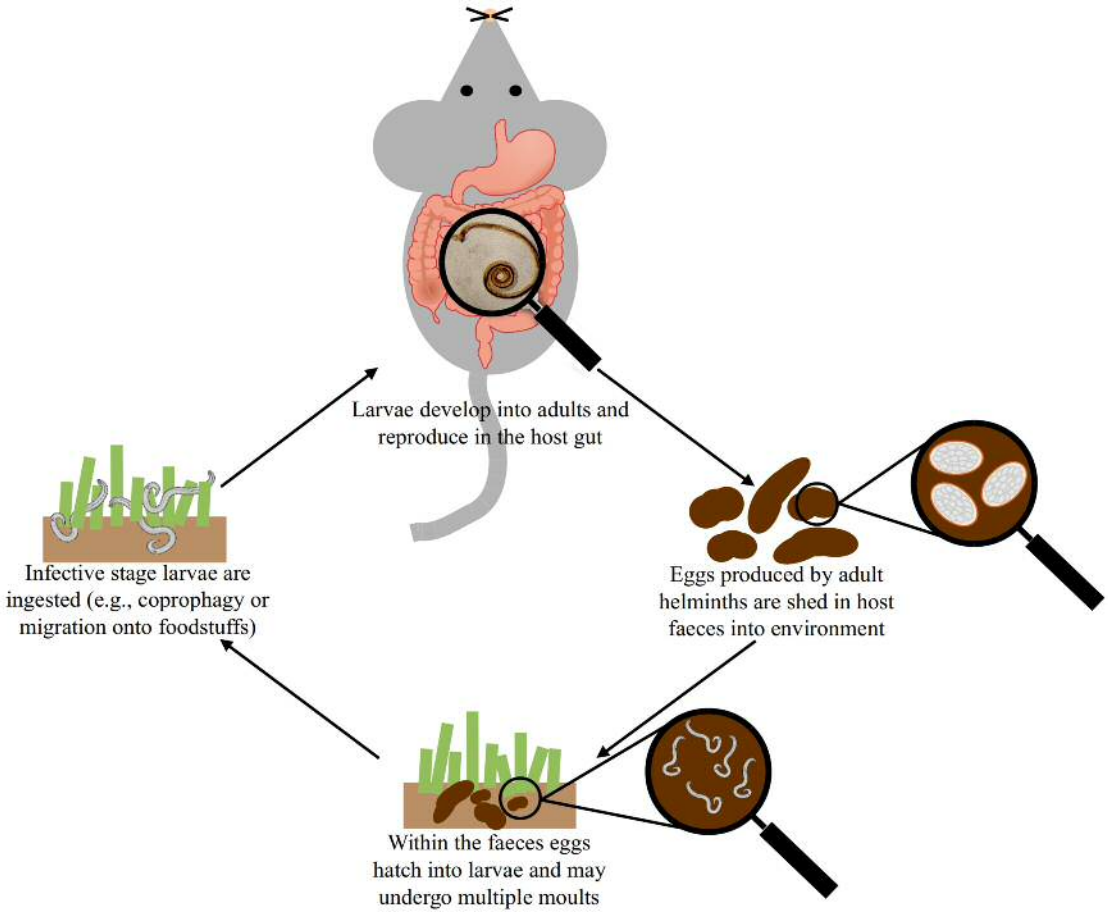

Figure 1.2: An overview of the life-cycle of a typical parasitic helminth that infects the gut. Generally, the life-cycle undergoes three separate stages: the egg, at least one larval stage and the adult stage, although not all of these life stages are necessarily parasitic, and may occur in the environment. 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 into adults, reproduce and shed 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 part of the gut biome. 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. While to date around ten papers review microbiota-helminth interactions (Bancroft *et al.*, 2012; Berrilli *et al.*, 2012; Glendinning *et al.*, 2014; Loke and Lim, 2015; Mutapi, 2015; Reynolds *et al.*, 2015; Gause and Maizels, 2016; Giacomin *et al.*, 2016a; Zaiss and Harris, 2016; Guernier *et al.*, 2017), these are largely conceptual, and rely on evidence from fewer than 25 studies which have directly investigated microbiota-helminth interactions (see Martínez-Gómez *et al.*, 2009; Hayes *et al.*, 2010; Walk *et al.*, 2010; Jensen *et al.*, 2011; Broadhurst *et al.*, 2012; Li *et al.*, 2012; Wu *et al.*, 2012; Coêlho *et al.*, 2013; Cooper *et al.*, 2013; Rausch *et al.*, 2013; Cantacessi *et al.*, 2014; Lee *et al.*, 2014; Osborne *et al.*, 2014; Reynolds *et al.*, 2014; Houlden *et al.*, 2015; Kreisinger *et al.*, 2015; McKenney *et al.*, 2015; Zaiss *et al.*, 2015; Cattadori *et al.*, 2016; Duarte *et al.*, 2016; Giacomin *et al.*, 2016b; Newbold *et al.*, 2017). However, evidence thus far indicates that the microbiota and parasitic helminths do interact (see Martínez-Gómez *et al.*, 2009; Hayes *et al.*, 2010; Walk *et al.*, 2010; Jensen *et al.*, 2011; Broadhurst *et al.*, 2012; Li *et al.*, 2012; Wu *et al.*, 2012; Coêlho *et al.*, 2013; Cooper *et al.*, 2013; Rausch *et al.*, 2013; Cantacessi *et al.*, 2014; Lee *et al.*, 2014; Osborne *et al.*, 2014; Reynolds *et al.*, 2014; Houlden *et al.*, 2015; Kreisinger *et al.*, 2015; McKenney *et al.*, 2015; Zaiss *et al.*, 2015; Cattadori *et al.*, 2016; Duarte *et al.*, 2016; Giacomin *et al.*, 2016b; Newbold *et al.*, 2017). For example, 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; Reynolds *et al.*, 2014), and in turn infection can influence the microbial community, usually by increasing bacterial diversity

(Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Rausch *et al.*, 2013; Reynolds *et al.*, 2014; Kreisinger *et al.*, 2015).

The majority of studies that have investigated or reviewed microbiota-macrobiota interactions have suggested that interplay between the immune system and gut microbiota is largely responsible for potential/observed interactions (Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Rausch *et al.*, 2013). Both microbiota and parasitic helminths have immunomodulatory effects on the host, and employ similar strategies to avoid host immune responses (Reynolds *et al.*, 2015). A long-accepted interaction between bacteria and parasites that occurs via the immune system is that which results from the $T_h1 - T_h2$ paradigm, whereby the T_h1 response, stimulated by microparasite (bacteria) infection is antagonistic to the T_h2 response initiated by a macroparasite (helminth) infection, and *vice versa* (Romagnani, 1997). Consequently, during a bacterial invasion the host may be more susceptible to a helminth infection, whilst the converse is also true, although it should be noted that this is a generalisation of much more complex immune response interactions (Romagnani, 1997). Immunomodulatory effects stimulated by microbiota and parasitic helminths also include the induction of regulatory T cells, which suppress host immune responses against both microbiota and macrobiota (see Faith *et al.*, 2011; Geuking *et al.*, 2011; Maizels and Smith, 2011). Evidence that both the microbiota and macrobiota can prevent autoimmune diseases (e.g., Wen *et al.*, 2008; McSorley and Maizels, 2012; Kostic *et al.*, 2013) has brought to light that these two communities each suppress the host immune response to allergens and autoantigens in a similar way, thus share cross-talk (see Reynolds *et al.*, 2015). Similarly, toll-like receptors, which recognise pathogens based on cell-surface molecules, can be disrupted by helminths, which in turn may influence the hosts response to microbiota changes and *vice versa* (see Reynolds *et al.*, 2015). In addition, the microbiota and macrobiota can interact via metabolic pathways, although it is

unclear if changes in metabolism associated with microbiota/macrobiota are affected by, or alternatively influence interactions. Microbiota-macrobiota interactions mediated by metabolism may also result from changes in nutrient absorption in the intestine associated with parasite infection, and/or from metabolite production by the parasite (Wang *et al.*, 2009; Li *et al.*, 2012; Houlden *et al.*, 2015).

Although changes in microbiota associated with helminth infection have been attributed to microbiota-immunity interplay (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 or in another intermediate host, and acquire their own microbiota (Walk *et al.*, 2010; Figure 1.2), which could be transmitted to the gut of the definitive host (Perkin *et al.*, 2014; 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 (Weinstein *et al.*, 1969). Consequently, some helminths are unable to form persistent infections in germ-free mice (also referred to as gnotobiotic; sterile or having a reduced and/or 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 on other components of the gut biome. The vast majority of research on microbiota-macrobiota interactions has been performed using laboratory animals. This is largely 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 with natural and intact microbiota and macrobiota composition.

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 in mature beech forests (*Fagus sylvatica* L.) with understorey, 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 (46°03'28.6"N, 11°00'47.9"E), 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 (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. A field experiment that examined the effect of helminth perturbation (by anthelmintic) on microbiota composition is presented (Chapter 3), 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, Figure 1.3). The effect of faecal microbiota on helminth development was then explored, whereby probability and rate of helminth egg development in microbiota of 'self' faeces from the original 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).

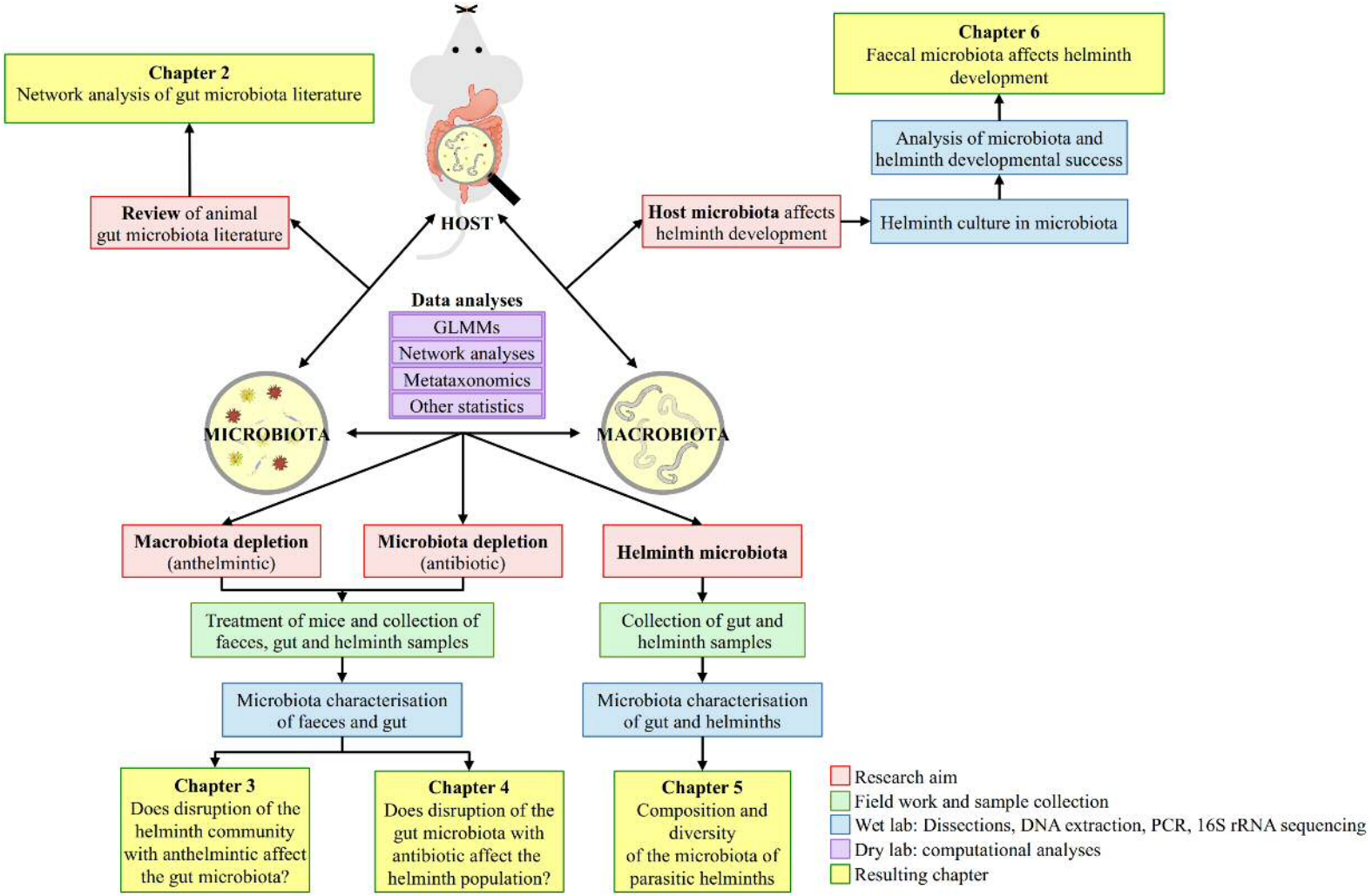

Figure 1.3: Overview of aims for each data chapter, including how samples were collected and analysed to answer each question posed. The thesis is composed of one literature review and four experimental chapters, of which three involved experimental manipulation of the microbiota and/or macrobiota, and one characterised the microbiota of host microbiota and the parasitic helminths therein. Biometric data was recorded for all individual mice studied, in addition to the collection of helminth and microbiota samples (e.g., for statistical analyses and 16S rRNA sequencing). Data were 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 has 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 focussing 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; Bright and Bulgheresi, 2010; Claesson *et al.*, 2012), and is vital to host 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 of research is starting to prosper, 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).

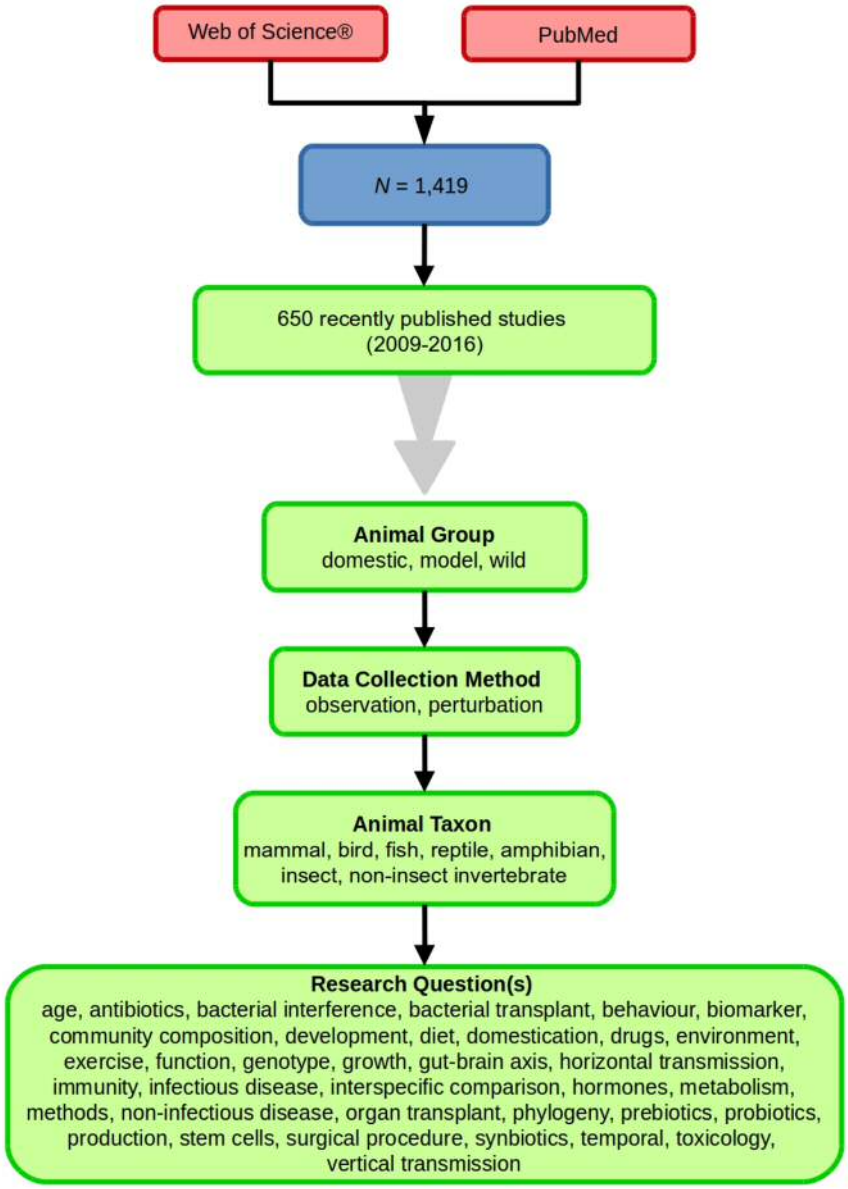

Figure 2.1: Work flow for categorising gut microbiota studies on non-human animals following searches in Web of Science® and PubMed. Of the 1,419 relevant articles identified, 650 recently published studies (2009-2016) were categorised into one of three animal groups (domestic, model or wild animals).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

2.2.3 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 an edge the co-occurrence of those questions within a scientific paper(s). Each network was constructed from an *n* by *n* symmetrical adjacency matrix; whereby a row and a column were present for each of the 36 research questions, and numbers within the matrix represented the total number of studies in which each pairwise combination of research questions co-occurred, in a given animal group. Numbers at the intercept of a given research question (at the diagonal centre) indicated the total number of studies in which that research question was addressed, regardless of whether it co-occurred with any other research questions. As the matrix was symmetrical, edges were non-directed, i.e., a link between any pairwise combination of research questions had the same value in both directions: for example, the nodes *i* to *j* had the same value as *j* to *i*. In each network graph, the size of each circle (node size; *s*) was weighted according to the total number of studies addressing that question, and the width of lines joining each circle (edge width) was weighted by the number of studies in which two given research questions co-occurred (Figure 2.2). 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.

2.2.4 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 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).

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.

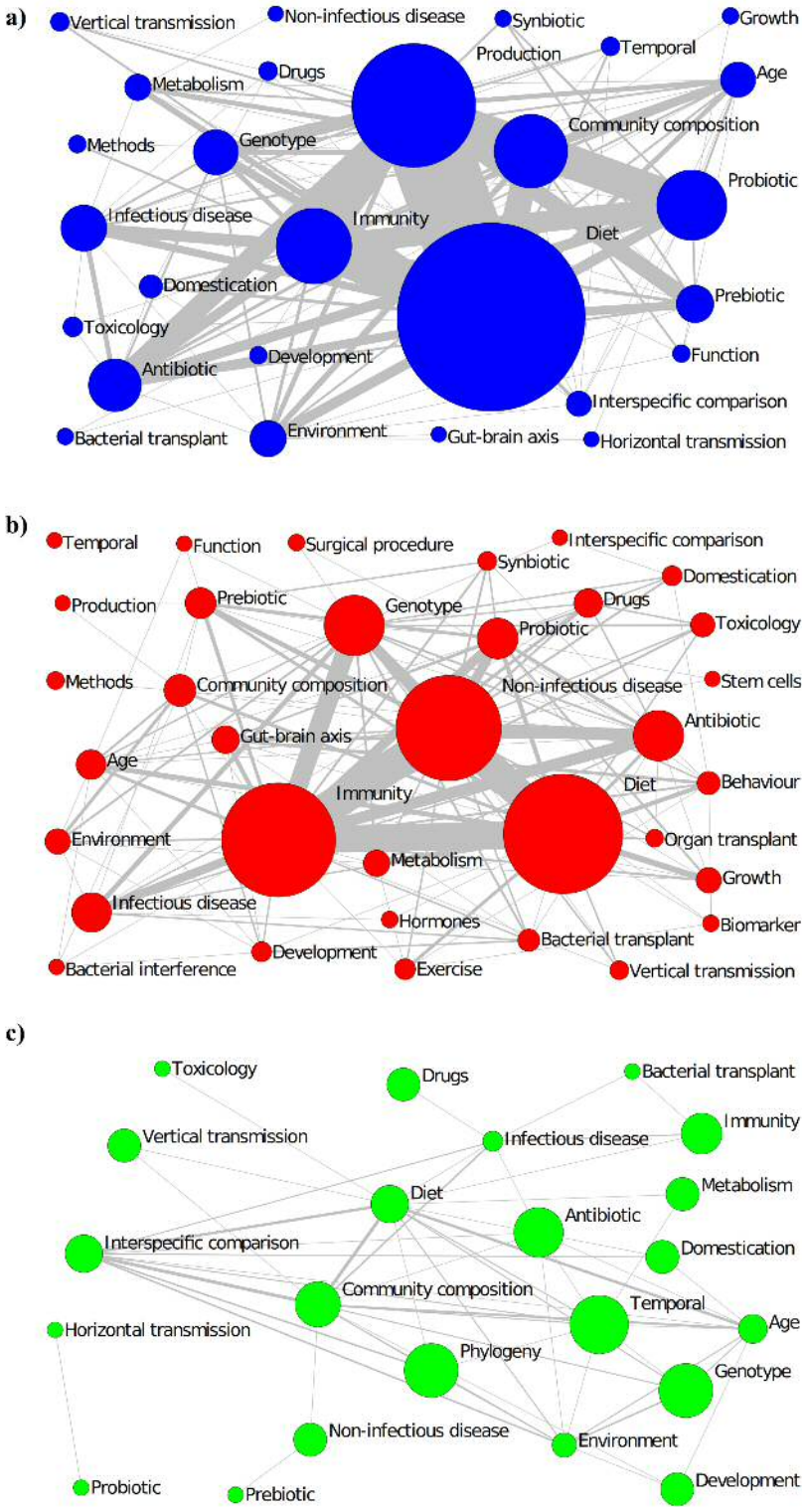

Figure 2.2: Network graphs illustrating the frequency of 36 research questions addressed by gut microbiota studies on a) domestic b) model and c) wild animals, and how frequently these questions co-occur within the 650 studies. Each node (circle) represents a research question, with diameter weighted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

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).

2.2.5 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).

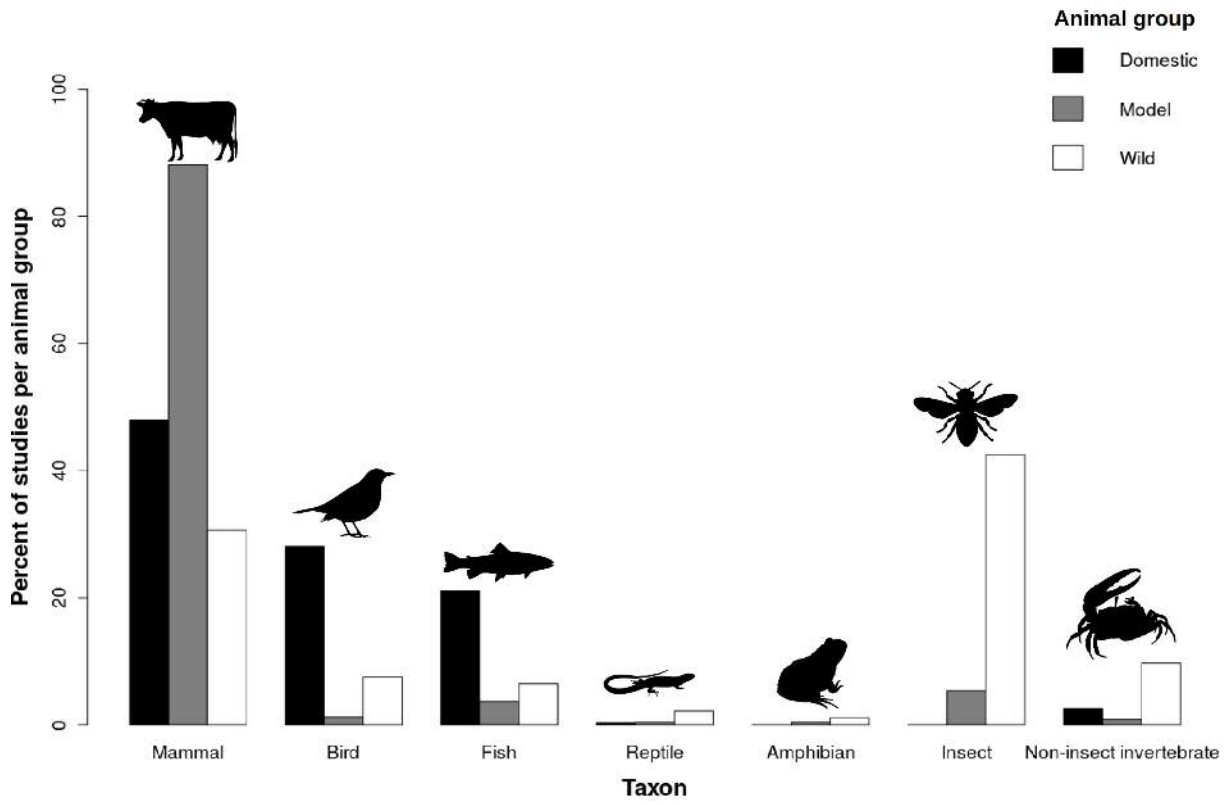

Figure 2.3: The percentage of gut microbiota studies within three animal groups; domestic (black), model (grey) or wild (white), investigating different animal taxa. For each animal group the combined percentage of studies across all taxa equate to 100% of studies for that group.

2.2.6 What are the research foci of animal microbiota?

'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 instead '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 have 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 that 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 $BC = 19.09 \pm 3.99$), than in domestic ($BC = 15.99 \pm 3.41$) or wild ($BC = 12.19 \pm 3.41$) 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 in order 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) 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, it is likely that interdisciplinarity will 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).

E.L.P: Conceived and designed study, collected, analysed and interpreted data, wrote manuscript.

H.C.H: Provided detailed comments on methods and interpretation of data, as well as all versions of the manuscript.

J.R.M: Provided comments on the final version of the manuscript.

S.E.P: Conceived and designed study, supervised network analyses, provided comments on the manuscript.

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

Helminth infection of the gut is associated with morbidity and economic loss, and anthelmintics are widely administered to humans, livestock, and companion animals to control infections. Although helminth resistance has been well studied, it is largely unknown if perturbation of the helminth community by an anthelmintic treatment has knock-on effects on other components 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-treatment in both the anthelmintic and the control group. Gut microbiota did not show significant taxonomical differences in composition associated with anthelmintic treatment, but faecal microbiota did (Bray Curtis: p <0.01; weighted UniFrac: 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, faeces, and gut microbiota of the small intestine, caecum and colon combined. The results demonstrate that although the abundances of some OTUs do significantly change between pre- and post-treatment, overall, gut microbiota composition is resilient to anthelmintic treatment, but faecal microbiota is not. Changes in faecal microbiota composition that were associated with anthelmintic treatment may have resulted from changes in host immune factors shed in faeces following a reduction in helminth infection load. 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 found here may have implications for helminth development.

3.2 Introduction

Billions of humans, as well as wildlife and livestock, harbour parasitic helminth infections of 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, such simple hygiene measures are not currently accessible worldwide. 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). During mass drug administrations, individuals are often indiscriminately treated with anthelmintic, regardless of whether or not there is evidence that they are infected (Truscott *et al.*, 2015). In addition, many anthelmintic products are available 'over-the-counter' and thus can be administered inappropriately and without professional medical or veterinary advice (Nielsen, 2009), factors which can all contribute to resistance of helminths to the currently available pharmaceutical treatments (Wolstenholme *et al.*, 2004). Furthermore, we do not know at present if anthelmintics affect 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 is usually associated with changes in microbial composition, which can occur in the gut 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 variation in the microbiota of the host stomach (Kreisinger *et al.*, 2015), while infection by 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 an established 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 could shed light on the more extensive effects of anthelmintic on the host, and in addition, also 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 administered 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 in turn 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.

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 (dominated by mature stands of *Fagus sylvatica* L.), and was situated at least 250 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), to identify individuals at subsequent recaptures. 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), while adults were categorised according to breeding condition (descended testes for males and perforated vagina or pregnant for females; after Gurnell *et al.*, 1990); individuals with adult pelage that were not in breeding condition were classified as sub-adults. Faeces that had accumulated overnight inside traps containing a single individual were collected and transported to the laboratory at 4°C. For 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 (after Dunn and Keymer, 1986) to calculate helminth eggs per gram of faeces (EPG), used as a proxy measure of helminth egg shedding. When an individual was captured more than once during a trapping week, subsequent faecal samples were collected for microbiota analyses, and upon returning to the laboratory were immediately frozen at -80°C until DNA extraction (see '*3.3.4 16S rRNA gene sequencing'* below). After occupation, traps were sterilised using sodium

hypochlorite (bleach), followed by 4% chlorhexidine solution (Nuova Farmec, Italy), re-baited and replaced. A total of 25 mice were randomly selected for sacrifice throughout the course of the experiment for gut microbiota and adult helminth analyses; three pre-treatment (Cavedine n = 3, Pietramurata n = 0) and nine post-treatment (Cavedine n = 5, Pietramurata n = 4) from the anthelmintic group, plus six pre-treatment (Cavedine n = 6, Pietramurata n = 0) and seven post-treatment (Cavedine n = 5, Pietramurata n = 2) from the control group. Animals selected for sacrifice were transported to the laboratory, whereupon they were euthanised by an overdose of isoflurane, followed by cervical dislocation, and immediately frozen at -80°C until dissection (see '*3.3.3 Analyses of gut samples*' below).

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 a 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 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). Between each administration of a treatment the gavage needle was sterilised using 4% chlorhexidine solution (Nuova Farmec, Italy). 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 adapted from 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 gut section were diluted with TBS and scanned for helminths at 10× magnification (Leica© MS5 microscope with a Leica© CLS100 light attachment). Faeces were homogenised in TBS and scanned for helminths at 10× magnification. Helminths were quantified and collected according to species, gut section and mouse individual in 70% ethanol in case of future analyses. 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 and faecal material using the following centrifugation steps: total contents of the tube were centrifuged for 950 G for 10 minutes at 4°C, resulting in a pellet containing the gut membrane and non-bacterial lumen contents (e.g., digested food). This pellet was discarded, but the supernatant was further centrifuged at 9000 G for 15 minutes at 4°C. The resulting supernatant was discarded and the remaining 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 (Cavedine n = 8, Pietramurata n = 7 individuals) from the anthelmintic group, and 19 samples from 8 individuals from the control group (Cavedine n = 1, Pietramurata n = 7 individuals). In addition, the bacterial pellets from the small intestine, caecum and colon samples (the microbiota of the stomach was not analysed) 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 bacterial pellet sample. 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). Recovered DNA was quantified using a Qubit 2.0 Fluorometer with a Qubit® dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA). The V3-V4 region (464 nucleotides) 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). 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 DNA extraction and PCR reactions were included, and genomic DNA from the Microbial Mock Community B (Staggered, Low Concentration), v5.2L (BEI Resources, Manassas, VA, USA) was also included in the sequencing library to assess the effect of data processing on observed community content. Purity and quality of PCR products were determined using a QIAxcel capillary electrophoresis system (Qiagen, Valencia, CA, USA). PCR products were purified using XP AMPure beads (Beckman Coulter Inc., Brea, CA, USA) and dual indices were attached by a second PCR (8 cycles) using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). The resulting libraries were pooled in an equimolar way to produce the final amplicon library, which was sequenced on an Illumina® MiSeq (PE300) platform (MiSeq Control Software 2.5.0.5 and Real-Time Analysis software 1.18.54.0) at the CIBIO Next Generation Sequencing Platform of the University of Trento, Trento, Italy.

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. Primers were trimmed from the resulting, merged 16S fragments, and fragments were then discarded if they had an average expected error (AvgEE) probability 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 helminth abundance and EPG

Generalised linear mixed models (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* spp. Due to a lack of power, differences in the abundances of the other two species identified, *T. muris* and *S. frederici,* were not analysed separately, but were included in total helminth abundance analyses; only a single *T. muris* infected one individual and 15 *S. frederici* in another individual were present in the anthelmintic group.

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* spp. A total of 118 FEC measurements were used for statistical analyses of EPG, which included at least one pre- and one post-treatment sample from any given individual; 63 FECs from 10 individuals in the anthelmintic group (Cavedine n = 5, Pietramurata *n* = 5) and 55 FECs from 14 individuals in the control group (Cavedine n = 6, Pietramurata *n* = 8). Due to a lack of statistical power, differences in *T. muris* and *S. frederici* EPG were not analysed separately but were included in total EPG analyses; only one *T. muris* egg and one *S. frederici* egg were present in faeces from the anthelmintic group. In each model, the response variable was abundance or EPG of either *H. polygyrus*, *Hymenolepis* spp.*,* or of all species combined. Host sex, host breeding status, host body mass, helminth diversity (total number of helminth species found in an

individual), treatment group (anthelmintic or control), treatment period (pre- or post-treatment) and the number of doses administered were explanatory variables. In addition, the model included the following two-way interaction terms as explanatory variables: treatment group with treatment period, and treatment group with number of doses administered, plus 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 random intercepts for each model. Statistical analyses used the package *glmmADMB*, version 8.3.3 (Fournier *et al.*, 2012; Skaug *et al.*, 2016). A process of multi-model inference was used to compare all possible models using the R package *MuMIn* (Bartoń, 2015) and the most parsimonious model was selected using a threshold of ΔAICc <2 (Burnham and Anderson, 2003).

3.3.7 Statistical analyses of microbiota - diversity

GLMMs were used to assess whether there was a significant association between microbiota alpha diversity and anthelmintic treatment. The inverse Simpson index was chosen to calculate alpha diversity as it is less affected by the presence of rare OTUs, (which frequently result from sequencing error, e.g., Wen *et al.*, 2017), indicates OTU richness with consistent evenness, and is also considered the most robust alpha diversity metric (e.g., compared to Shannon index; DeJong, 1975; Gihring *et al.*, 2012). Preliminary analyses indicated that data had insufficient power to include treatment and treatment period (anthelmintic and control data pooled) as a two-way interaction 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 microbiota alpha diversity between the anthelmintic and control group, to ensure changes between pre- and post-treatment individuals in each of the two groups were comparable. When this assumption was met, separate GLMMs for the anthelmintic and control group 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 and sampling month were each modelled as a nested random intercept for each model.

3.3.8 Statistical analyses of microbiota - composition

A distance-based redundancy analysis (db-RDA; capscale function in R package *vegan*; Oksanen *et al.*, 2017) 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) and faeces. The db-RDA performs constrained ordinations, but unlike most other methods of constrained ordination, uses non-Euclidean distance measures (data which has >2 dimensions, e.g., OTU abundance tables). Here, distance matrices of microbiota data (OTU abundance tables) were calculated using Bray–Curtis dissimilarities (i.e., compositional dissimilarity indices that account for proportional differences in OTUs among samples) and weighted UniFrac dissimilarities (which account both for proportional differences in OTUs and their phylogenetic relatedness; Lozupone and Knight, 2005). OTU abundance 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. For each dissimilarity matrix a constrained analysis of principal coordinates (CAP) was performed, which tested whether changes in microbiota composition were associated with environmental variables (i.e., anthelmintic treatment). The resulting eigenvalues were visualised on an ordination plot, with ordinations starting at [0,0], for optimal and consistent visualisation. Significance (p <0.05) of the effect of environmental variables on the ordination was assessed using permutation-based ANOVA tests on the constrained axes.

3.3.9 Statistical analyses of microbiota - OTU abundances

To determine how OTU abundances differed 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 in *DESeq2*, and significance values (p <0.05) were derived using likelihood-ratio tests (Anders and Huber, 2010; Love *et al.*, 2014).

3.4 Results

3.4.1 The effect of anthelmintic on helminth abundance

Anthelmintic treatment efficacy was assessed using helminth prevalence and abundance data. Four helminth species; *H. polygyrus*, *Hymenolepis* spp., *S. frederici* and *T. muris*, were isolated from mouse guts, however the prevalence and abundance of *S. frederici* and *T. muris* were insufficient for separate analyses (Table 3.1, see Appendix A.3, Figure A.3.1 for boxplots of analysed helminth abundance data). Prevalence of *H. polygyrus* and *Hymenolepis* spp. were both lower in post- compared to pre-anthelmintic treated individuals (Table 3.1; Figure 3.1). Anthelmintic treatment was not associated with a significant change in overall helminth abundance (d.f. = 9, Z = -1.59, p = 0.11), nor in the abundance of *H. polygyrus* (d.f. = 10, Z = -1.07, p = 0.29), however, *Hymenolepis* spp. abundance decreased by 97.2% between pre- and post-treatment individuals (d.f. = 8, Z = -2.13, p = 0.03; Table 3.1; Figure 3.1). There was no significant difference in overall helminth abundance (d.f. = 10, Z = -0.64, p = 0.52), nor in the

abundances of *H. polygyrus* (d.f. = 8, Z = -0.84, p = 0.40) or *Hymenolepis* spp. (d.f. = 9, Z = 0.70, p = 0.49) in the control group (Table 3.1; Figure 3.1).

Table 3.1: Mean abundance (± standard error of mean) of helminths isolated from the gut of pre- or post-treatment individuals in an anthelmintic or control group.

Helminth species	Helminth abundance			
	Anthelmintic		Control	
	Pre	Post	Pre	Post
Total	153.0 ± 143.0	7.0 ± 1.4	75.3 ± 43.9	25.1 ± 10.7
H. polygyrus	6.3 ± 0.7	2.8 ± 0.8	11.7 ± 5.0	7.3 ± 1.6
Hymenolepis spp.	141.7 ± 138.7	4.0 ± 1.4*	10.2 ± 4.8	17.9 ± 11.2
S. frederici	5.0 ± 5.0	0.1 ± 0.1	53.5 ± 41.0	0
T. muris	0	0.1 ± 0.1	0	0

* *Represents a significant decrease in helminth abundance between pre- and post-treatment individuals in either an anthelmintic or control group.*

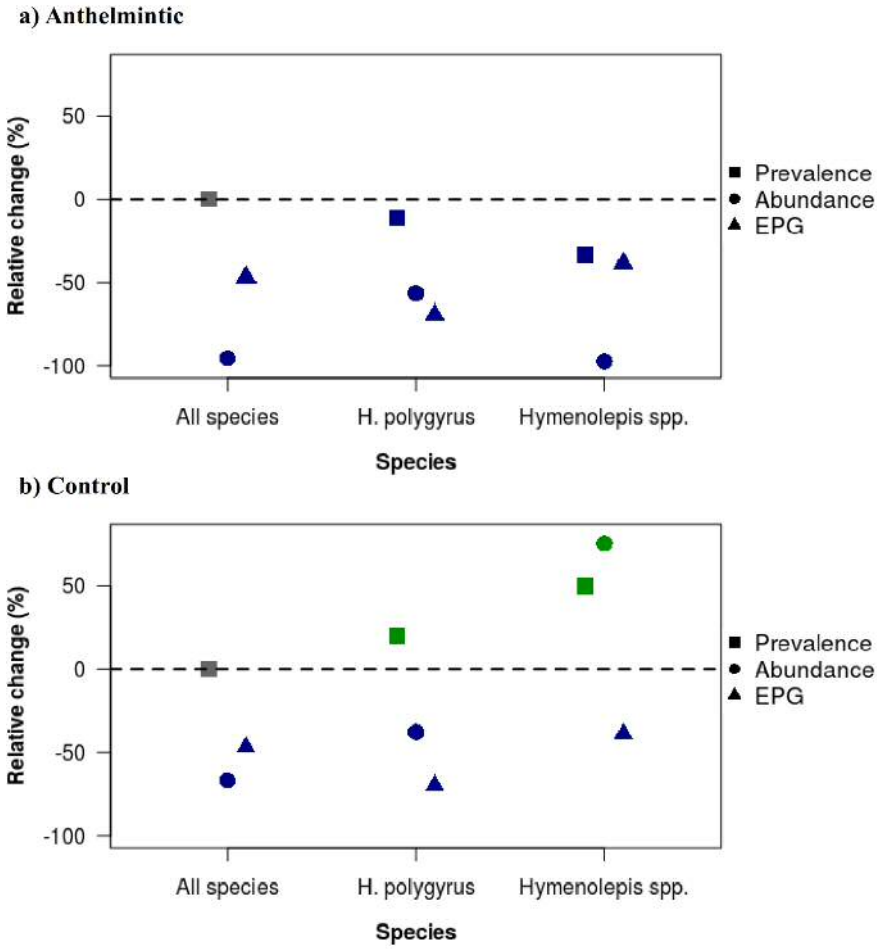

Figure 3.1: Relative changes (%) in helminth prevalence, abundance and eggs per gram (EPG) of faeces between pre- and post-treatment individuals in an a) anthelmintic and b) control group for all helminth species, *Heligmosomoides polygyrus* and *Hymenolepis* spp. Prevalence, abundance and EPG of other 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.

3.4.2 The effect of anthelmintic on helminth EPG

Eggs from *H. polygyrus*, *Hymenolepis* spp., *S. frederici* and *T. muris* were identified in mouse faeces, however the prevalence and EPG of both *S. frederici* and *T. muris* eggs were insufficient for individual analyses (Table 3.2; see Appendix A.3, Figure A.3.2 for boxplots of analysed helminth EPG data). There was no significant change in helminth egg shedding in faeces between pre- and post-anthelmintic treatment (d.f. = 58, Z = -0.35, p = 0.73; Figure 3.1; Table 3.2). Similarly, egg shedding of *H. polygyrus* (d.f. = 58, Z = -0.12, p = 0.90) and *Hymenolepis* spp. (d.f.

= 58, Z = -1.21, p = 0.23) did not significantly change post-anthelmintic treatment (Figure 3.1; Table 3.2). In the control group there was no significant change in total egg shedding (d.f. = 51, Z = 0.75, p = 0.45), nor in *H. polygyrus* (d.f. = 51, Z = -0.55, p = 0.58) and *Hymenolepis* spp. egg shedding (d.f. = 51, Z = 0.58, p = 0.56) between pre- and post-treatment individuals (Figure 3.1; Table 3.2).

Table 3.2: Mean number of helminth eggs per gram (EPG) of faeces (± standard error of mean) in faecal samples collected from pre- or post-treatment individuals in an anthelmintic or control group, used as a proxy measure for helminth egg shedding.

Helminth species	Helminth EPG			
	Anthelmintic		Control	
	Pre	Post	Pre	Post
Total	1,076.7 ± 500.8	574.1 ± 148.3	546.5 ± 223.2	814.3 ± 246.8
H. polygyrus	290.0 ± 123.9	88.5 ± 38.1	207.6 ± 98.3	172.0 ± 68.4
Hymenolepis spp.	786.7 ± 449.1	483.8 ± 147.3	317.8 ± 212.6	622.1 ± 252.2
S. frederici	0	0.9 ± 0.9	0	0
T. muris	0	0.9 ± 0.9	21.2 ± 21.2	17.7 ± 13.0

3.4.3 The effect of anthelmintic on gut and faecal microbiota diversity

Of the sequenced samples, reads from two faecal, one small intestine, one caecum and one colon sample 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. Anthelmintic treatment did not affect gut microbiota alpha diversity; inverse Simpson indices for microbiota of the small intestine (d.f. = 6, Z = -1.70, p = 0.09), caecum (d.f. = 7, Z = -0.82, p = 0.41), colon (d.f. = 7, Z = 0.37, p = 0.71) and faeces (d.f. = 32, Z = -1.83, p = 0.07) were not significantly different between pre- and post-treatment individuals (Table 3.3; Figure 3.2). Similarly, in the control group there were no significant differences in microbiota alpha diversity of the caecum (d.f. = 7, Z

= 0.77, p = 0.44), colon (d.f. = 10, Z = -0.06, p = 0.96), or faeces (d.f. = 14, Z = 0.22, p = 0.82) between pre- and post-treatment individuals (Figure 3.2). The small intestine was the only exception; microbiota alpha diversity was significantly higher in post- compared to pre-treatment individuals in the control group (d.f. = 10, Z = 2.71, p <0.01; Table 3.3; Figure 3.2).

Table 3.3: Mean inverse Simpson index (± standard error of mean) for alpha diversity of microbiota in each sampled gut section from pre- or post-treatment individuals in an anthelmintic or control group.

Gut section	Mean inverse Simpson index (± standard error)			
	Anthelmintic		Control	
	Pre	Post	Pre	Post
Small intestine	28.7 ± 15.8	9.0 ± 4.7	4.6 ± 1.8	9.3 ± 3.0†
Caecum	22.9 ± 9.6	31.0 ± 6.0	29.8 ± 6.6	37.2 ± 4.7
Colon	31.4 ± 13.0	32.1 ± 6.5	33.9 ± 3.5	34.3 ± 5.5
Faeces	37.3 ± 4.5	23.4 ± 3.4	36.4 ± 5.0	37.2 ± 4.6

† Represents a significant increase in mean inverse Simpson Index between pre- and post-treatment individuals in either an anthelmintic or control group.

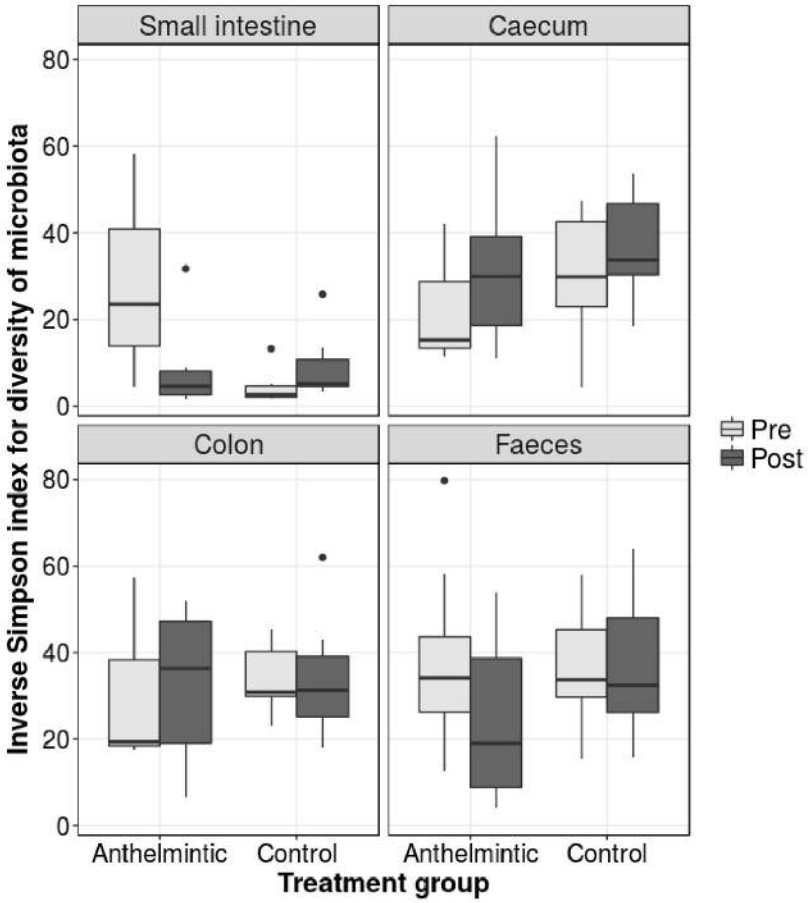

Figure 3.2: Inverse Simpson diversity index for alpha diversity of microbiota at three different sites within the gut (small intestine, caecum and colon), and faeces, for pre- and post-treatment individuals in an anthelmintic or control group. Boxes demonstrate the upper and lower quartiles of alpha diversity, with median alpha diversity indicated. Bars represent the minimum and maximum range of alpha diversity.

3.4.4 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 3.3).

The taxonomic composition of whole gut microbiota (i.e., small intestine, caecum and colon combined) changed significantly following anthelmintic treatment when measured by Bray-Curtis (d.f. = 66, F = 1.63, p <0.01), but not weighted UniFrac (d.f. = 66, F = 1.34, p = 0.19) dissimilarities (Figure 3.4). 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 3.5), 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 3.5). However, anthelmintic treatment did have a significant effect on colon microbiota composition, but only according to weighted UniFrac dissimilarities (Bray-Curtis: d.f. = 19, F = 1.15, p = 0.13; weighted UniFrac: d.f. = 19, F = 2.34, p = 0.02; Figure 3.5). 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 3.6).

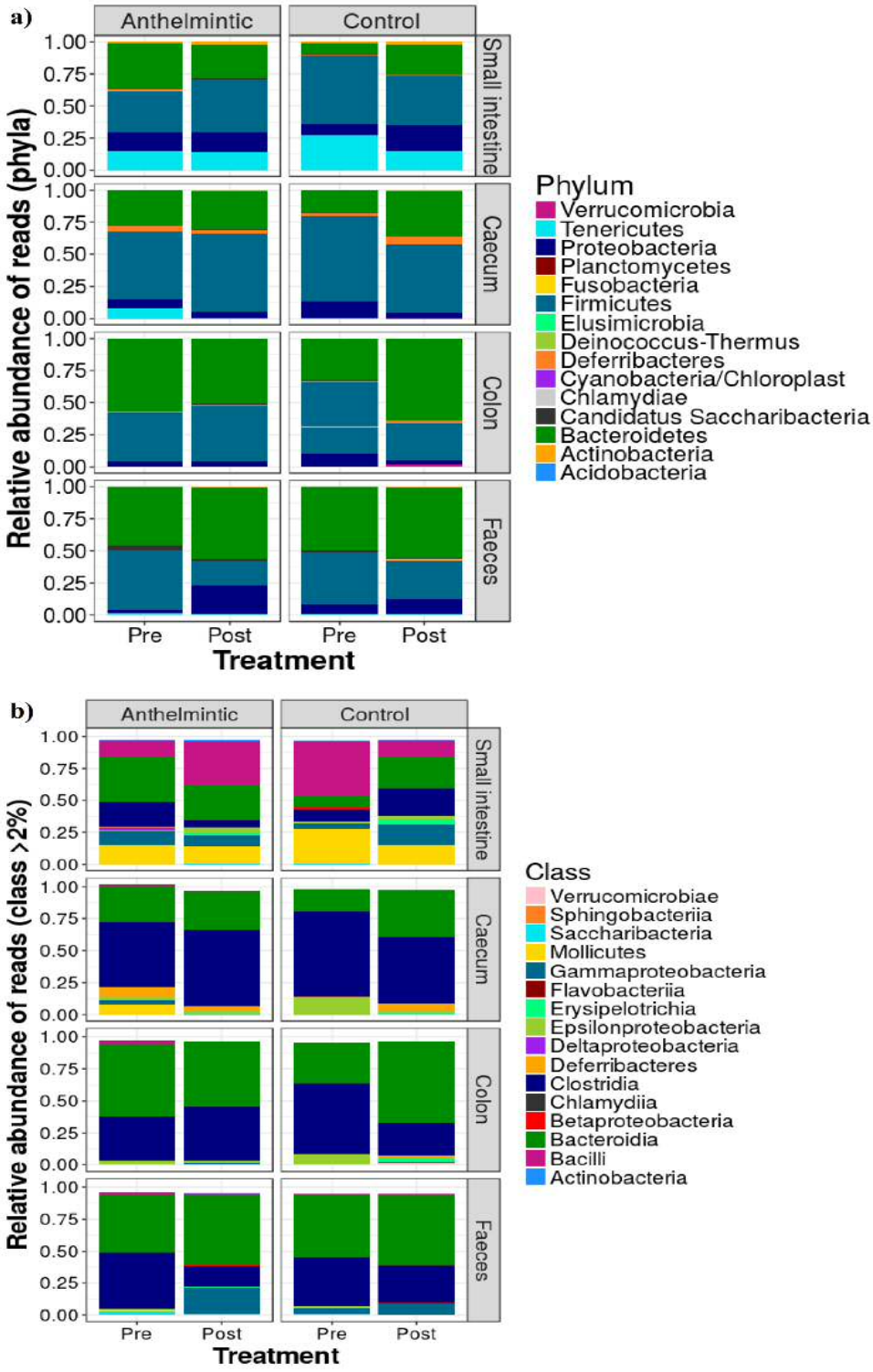

Figure 3.3: Mean relative abundance of bacterial a) phyla and b) classes (consisting >2% reads) present in the small intestine, caecum, colon and faeces of pre- and post-treatment mouse individuals in an anthelmintic or control group.

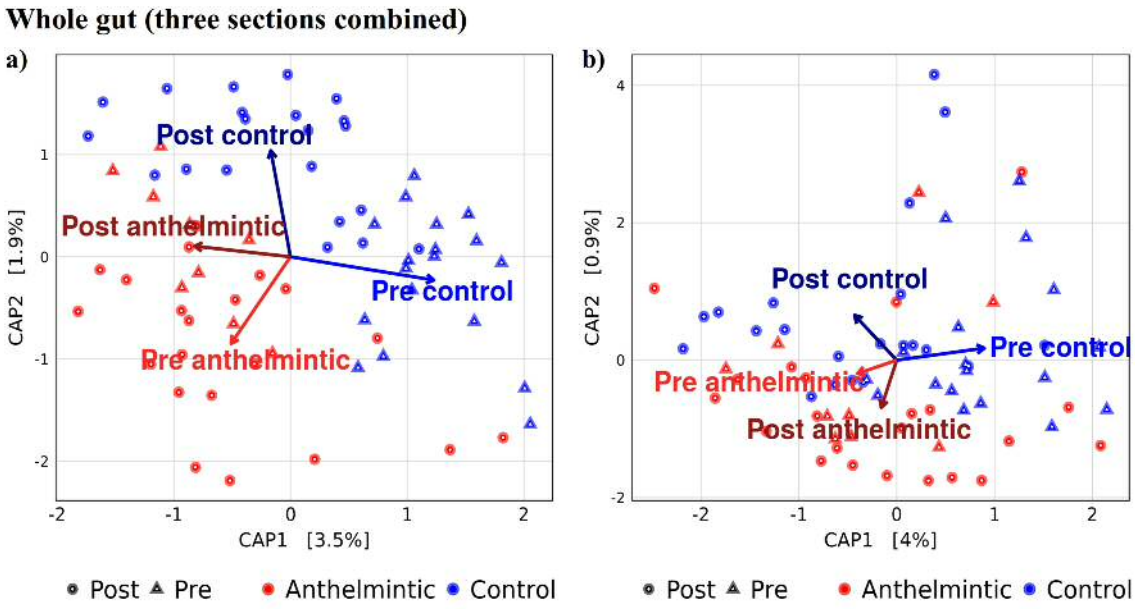

Figure 3.4: Ordination plots of divergence of microbiota taxonomic composition between samples of three gut sections (small intestine, caecum and colon) associated with either anthelmintic treatment or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution 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.

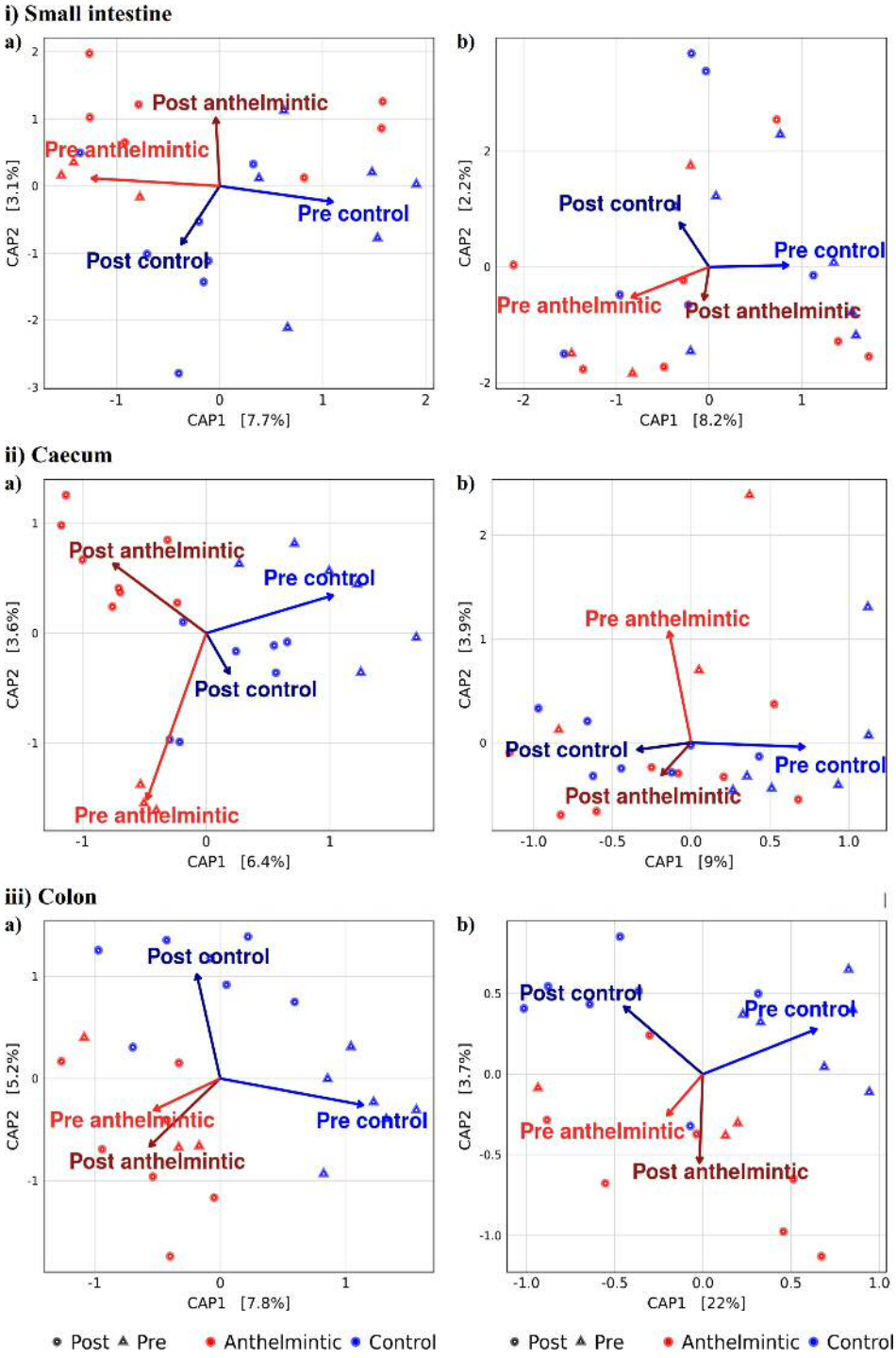

Figure 3.5: Ordination plots of divergence of microbiota taxonomic composition between i) small intestine, ii) caecum and iii) colon samples, associated with either anthelmintic treatment or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples 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.

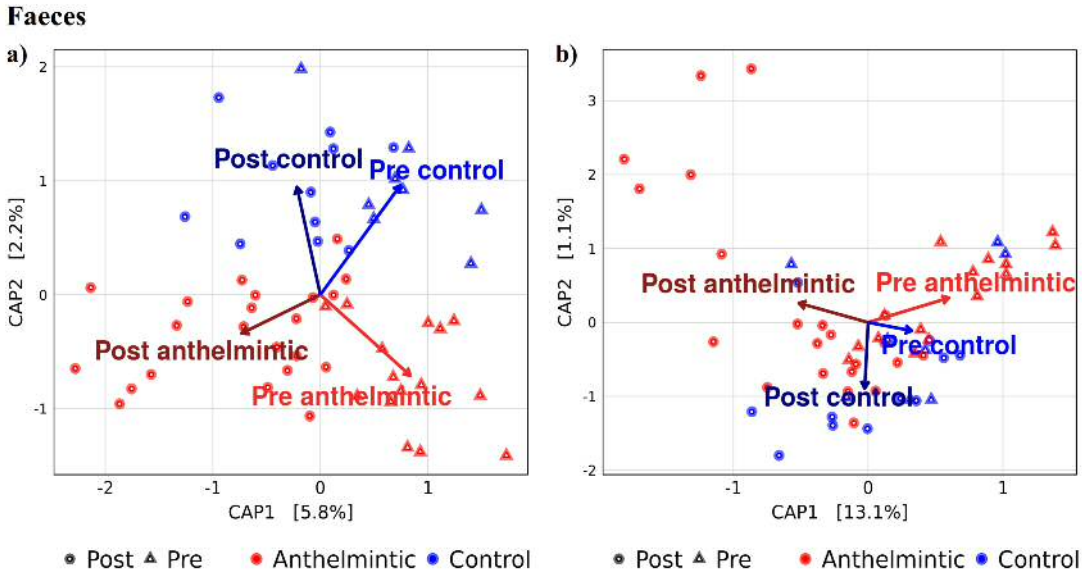

Figure 3.6: Ordination plots of divergence of microbiota taxonomic composition between faeces samples associated with either anthelmintic treatment or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation are shown. The length of the arrow indicates the relative importance of each treatment.

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

For whole gut microbiota, differences in OTU abundance between pre- and post-treatment individuals in the anthelmintic group were analogous to those in the control group (Figure 3.7; see Appendix A.3 and tables therein for detailed statistics). For example, the abundance of certain OTUs within the classes Clostridia, Deltaproteobacteria and Bacteroidia was higher in post- compared to pre-treatment individuals in both groups (Figure 3.7; see Appendix A.3, Table A.3.1 and A.3.2). In the caecum, anthelmintic treatment affected the abundance of OTUs from just two bacterial classes, which both decreased in abundance; Clostridia and Mollicutes (see Appendix A.3, Table A.3.4 and A.3.5). In the anthelmintic group, faecal microbiota showed substantial changes in OTU abundances between pre- and post-treatment individuals; OTUs from nine bacterial classes were significantly affected post- treatment, compared to just two bacterial classes

(Clostridia and Gammaproteobacteria), which both showed similar changes in abundance as in the anthelmintic group in the control group (Figure 3.7; see Appendix A.3, Table A.3.7 and A.3.8). No OTUs in either the small intestine or the colon changed significantly in abundance between pre- and post-anthelmintic treatment.

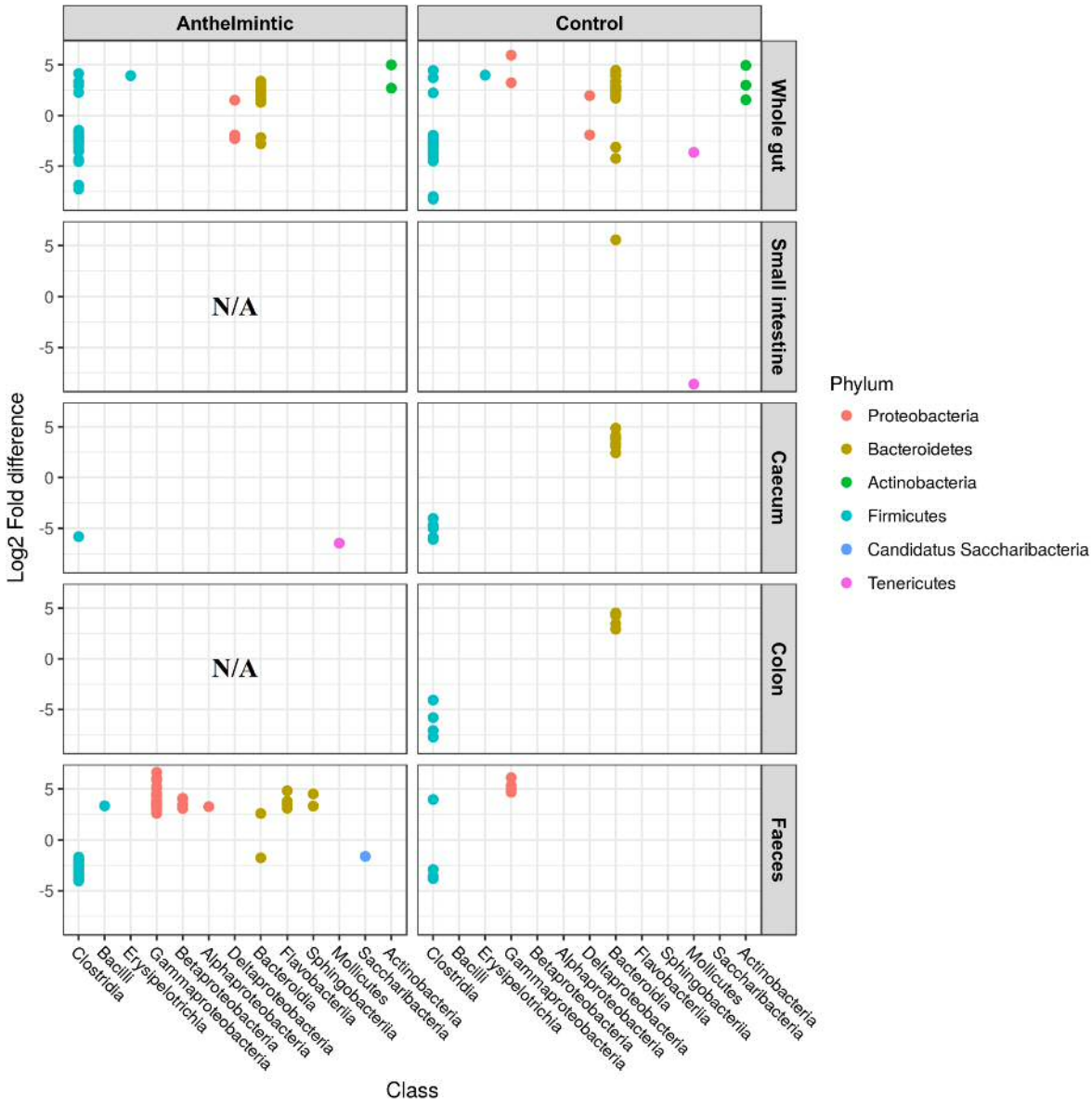

Figure 3.7: Bacterial OTUs in microbiota that were significantly different in abundance in post-treatment compared to pre-treatment individuals in an anthelmintic treatment or control group. Microbiota of the whole gut (small intestine, caecum, colon combined), small intestine, caecum, colon and faeces were analysed. OTUs are grouped by microbial class and coloured according to phylum. 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 there were no significant changes in abundance of any OTUs between pre- and post-treatment individuals.

3.5 Discussion

Anthelmintic treatment did not affect the alpha diversity of microbiota (Figure 3.2), but did have a significant effect on microbiota taxonomic composition of the colon and faeces, and when all three gut sections where considered together (Figure 3.3 - 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). Together, these results suggest that changes in microbiota associated with anthelmintic treatment were either driven by changes in the abundances of bacteria already present in the gut, or the net loss and gain of different bacterial OTUs associated with anthelmintic treatment remained constant.

In the current study there was a significant change in taxonomic composition of faeces following anthelmintic treatment (Figure 3.5), and OTUs from four phyla changed significantly in abundance in these samples (Figure 3.6). All OTUs, barring one from the phylum Bacteroidetes, increased post-anthelmintic treatment. Despite including sampling month as a random intercept in all statistical analyses, as the study was conducted over the course of four months it is possible that changes in microbiota and OTU abundances resulted from natural seasonal variation (Maurice *et al.*, 2015), and not necessarily anthelmintic treatment. However, in support of the possibility that anthelmintic treatment was responsible for faecal microbiota changes, Houlden *et al.*, (2015) also observed increases in Bacteroidetes abundance (and diversity) following anthelmintic treatment. Interestingly, the opposite 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 were investigated, as well as the anthelmintic used (Cooper *et al.*, 2013; Sirois, 2013; Houlden *et al.*, 2015).

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), are able to colonise more successfully during helminth infection, due to immune system suppression (Steenhard *et al.*, 2002) and tissue damage (Murray *et al.*, 1970), and these bacteria could 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 resulting impact on microbiota.

Notably, there was a significant increase in the alpha diversity of the small intestine microbiota between pre- and post-treatment individuals in the control group (Table 3.3; Figure 3.2), but no such change was observed in the anthelmintic group. As individuals in the control group were administered a sham gavage of ultra-pure water using a gavage needle which was sterilised between each use, it is unlikely (although not impossible) that there was a subsequent introduction

of bacteria into the small intestine associated with the control sham treatment. Instead, it is more likely that the significant differences observed in microbiota diversity in the control group were due to stochastic factors related to small sample sizes (pre-treatment $n = 3$; post-treatment $n = 6$), were a consequence of stress related to repeated animal trapping and handling (e.g., see Bangsgaard Bendtsen *et al.*, 2012; Le Floc'h *et al.*, 2014), and/or were a result of natural seasonal variation driven largely by changes in the availability of different food items (Maurice *et al.*, 2015). If the latter were true, it would be tempting to speculate that, as there was no significant difference in microbiota diversity between pre- and post-treatment individuals, nor in OTU abundances in small intestine or colon microbiota in the anthelmintic group, anthelmintic treatment may have a modulatory effect on microbiota, such that natural seasonal variation in the microbiota is inhibited. However, with the small samples sizes of the current study it is not possible to reliably make such statements.

Previous studies on the effect of anthelmintic treatment on microbiota have yielded mixed results; one study reported that the microbiota of faeces from individuals experimentally infected with helminths was 'restored' to a microbial community more similar to uninfected individuals (Houlden *et al.*, 2015), while another study did not observe significant changes in microbiota following anthelmintic treatment (Cooper *et al.*, 2013). The results of the current chapter were not as dramatic as those of Houlden *et al.*, (2015), and instead more closely resemble those observed by Cooper *et al.*, (2013), with anthelmintic treatment associated with very few significant changes in host microbiota. However, there are limited comparisons that can be made between the studies that have investigated the effect of anthelmintic on microbiota due to differences in study design. For example, in the Houlden *et al.*, (2015) study, helminth infection was experimental, and the model system was a laboratory rodent, while in Cooper *et al.*, (2013) treated patients harboured a

single species parasite infection, neither of which represent the same complexities of microbiota and macrobiota interactions as represented by the wild, replete system studied here (Amato, 2013). However, a strength of the Cooper *et al.*, (2013) study which could not be achieved here due to high parasite prevalence, is that uninfected individuals were also treated with anthelmintic, which allows us to tease apart the effect of the properties of the anthelmintic itself versus the act of helminth removal.

Whilst faecal samples were collected as a time series for each individual, evidence suggests that faeces from laboratory mice are not a reliable proxy for microbiota elsewhere in the gut (Pang *et al.*, 2012), and it should further be noted that faecal samples used in the current study were exposed overnight to potential contaminants in the field. In an effort to reconcile the limitations associated with faecal sample use, microbiota within different gut sections were also sampled and analysed, however, due to the destructive nature of gut sampling it was not possible to create a time series of gut microbiota samples from any given individual, and as such the 'pre' and 'post' data from gut samples were from unmatched individuals (with a bias towards individuals from Cavedine, due to small population sizes at Pietramurata), which may have naturally varied from one another, regardless of treatment. Furthermore, despite every effort to treat and sample animals at consistent time intervals, this was not always possible due to the unpredictable nature of capturing wild animals, and the resulting data provide evidence of a response to anthelmintic at a non-standardised time point. Consequently, results presented here should be interpreted with caution and future studies should aim to improve sample sizes and consider the benefits that an external rodent enclosure may provide (e.g., improved recapture rates).

There is widespread and often ungoverned use of anthelmintics in humans, livestock and companion animals (Vlassoff *et al.*, 2001; Nielsen, 2009; Vercruysse *et al.*, 2012), but the current study suggests that host microbiota can remain mostly stable following anthelmintic treatment. It is no surprise that anthelmintic did not directly affect host microbiota; although the avermectin family of anthelmintics (which includes ivermectin) have demonstrated 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), avermectins have yielded limited positive results in their ability to affect 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 them paralysed (Wolstenholme and Rogers, 2005). However, these ion channels are only present in protostome invertebrate phyla (Wolstenholme, 2012), and bacteria are not affected by this mechanism. Results from the present study also indicate that anthelmintic largely does 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). However, given that the World Health Organisation has committed to increase the percentage of children treated with anthelmintic to 75% by 2020 in areas where helminth infection prevalence is greater than 20% (Truscott *et al.*, 2015), it is important to consider that bacterial composition of microbiota did significantly change in some gut sections (colon and faeces) following anthelmintic treatment, and even comparatively small changes in microbiota composition can influence host health and *vice versa* (Bongers *et al.*, 2014; Sun and Kato, 2016).

To date, only the current study, and three others (Cooper *et al.*, 2013; Sirois, 2013; Houlden *et al.*, 2015) have investigated the affect 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 the effect of anthelmintic on the microbiota 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 experimentally 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 stable following anthelmintic treatment, and reduction in helminth infection. The results presented here support 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 affect microbiota. For example, 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; see also 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 have limited short-term impacts on the microbiota, mostly of the faeces, 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).

E.L.P: Designed study, collected, analysed and interpreted data, wrote manuscript.

J.K: Involved in designing study, provided guidance regarding appropriate analyses for microbiota data.

M.C: Provided guidance regarding processing of microbiota samples/data collection.

H.C.H: Involved in conceiving the study and organising field work and logistics, provided comments on each version of the manuscript.

J.R.M: Provided comments on the manuscript.

S.E.P: Conceived and designed study, involved in data interpretation, provided comments on each version of the manuscript.

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 administered to humans and animals due to their ability to prevent and treat bacterial infections, and induce growth in livestock. Although a diverse bacterial community shares the gut niche with other micro- and macro-organisms, the effect of antibiotic treatment on other components of the gut biome, such as the parasitic helminths, has been given little regard. Here, the effect of antibiotic on the helminth community was investigated in a wild, naturally infected rodent host. Antibiotic treatment did not significantly effect helminth abundance. However, fecundity of both *Heligmosomoides polygyrus* and *Hymenolepis* spp. significantly increased; egg shedding increased by 362% (p <0.01) and 2,165% respectively (p = 0.03), but there was no difference between pre- and post-treatment individuals for *in utero* eggs/μm^2. There was no difference in *H. polygyrus* size, however *Hymenolepis* spp. were 229.5% larger in post- compared to pre-treatment individuals (p <0.01). The results suggest that antibiotic treatment of the host increases absolute (but not net) helminth egg production. Increased egg shedding associated with antibiotic treatment may be a result of competitive release from bacteria or changes in the expression of genes within the host that protect against helminth infection. The implications of increased egg shedding following antibiotic treatment could include higher numbers of helminth eggs present in the environment, leading to increased rates of helminth transmission in the host 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). They are also exploited for their growth-inducing properties in livestock (Goossens *et al.*, 2005). However, 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 persist years after administration (Kilkkinen *et al.*, 2002; Hawrelak and Myers, 2004; Jernberg *et al.*, 2007; Jakobsson *et al.*, 2010). In addition, over- and inappropriate use of antibiotics have led to an alarming rate of antibiotic resistance in many strains of pathogenic 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 use remains widespread in both humans and animals; in Chile alone hundreds of tonnes of antibiotics are used annually only within the salmon farm industry (Landers *et al.*, 2012; Cabello *et al.*, 2013; Versporten *et al.*, 2014), while the annual worldwide antibiotic consumption of humans is 70 billion standard units (where one 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 despite this excessive use of antibiotics, we still do not know the full extent of how disrupting gut bacteria may affect the other components of the gut biome.

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 between these two communities are likely to be highly complex (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 observable both up- and down-stream from the site of helminth infection (Kreisinger *et al.*, 2015; McKenney *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 microbiota composition by antibiotic is also likely to affect the helminth community.

There is already evidence that antibiotics affect the helminth community and were tested as a possible treatment for helminth infections 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% (Wells, 1951; Brown, 1952). In addition, helminths that remained within the host following antibiotic administration were smaller in size, while fecundity and virulence were also 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; for example, administration of neomycin, dihydrostreptomycin and chloramphenicol resulted in increased helminth abundance (Wells, 1952a). The majority of these studies were performed before the advent of metataxonomic analyses, thus did not associate specific changes in microbiota with changes in the helminth community.

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, parasite establishment is less successful following antibiotic treatment, since 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). However, until now studies on the affect of antibiotic on helminths have used laboratory model organisms infected with a single helminth species, thus are unable to assess how a replete helminth community with interspecific interactions (Lello *et al.*, 2004; Telfer *et al.*, 2010) responds to antibiotic.

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.

4.3 Materials and methods

4.3.1 Study area and small rodent sampling

Live-trapping of *Apodemus flavicollis* was conducted following methods in Chapter 3 ('*3.3.1 Study area and small rodent sampling*'). Briefly, Ugglan multi-capture traps (Ugglan Type 2;

Grahnab, Sweden) were arranged in four grids of 64 traps each (8×8). 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). 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 capture, mice were processed following methods in Chapter 3 (‘*3.3.1 Study area and small rodent sampling*’), whereby individuals were tagged with a subcutaneous passive integrated transponder (Trovan ID 100; Ghislandi and Ghislandi, Italy), and host body mass, sex and breeding status were recorded. Faeces that had accumulated overnight inside traps occupied by a single individual were collected, and transported to the laboratory at 4°C. During 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 (after Dunn and Keymer, 1986) to calculate helminth eggs per gram of faeces (EPG). When an individual was

captured more than once during a trapping week, subsequent faecal samples were collected for microbiota analyses, which, upon returning to the laboratory were immediately frozen at -80°C until DNA extraction (see '*4.3.4 16S rRNA gene sequencing'* below). A total of 26 mice were randomly selected throughout the course of the experiment for gut microbiota and adult helminth analyses; six pre-treatment (Cavedine *n* = 6, Pietramurata *n* = 0) and seven post-treatment (Cavedine *n* = 6, Pietramurata *n* = 1) from the antibiotic group, plus six pre-treatment (Cavedine *n* = 6, Pietramurata *n* = 0) and seven post-treatment (Cavedine *n* = 5, Pietramurata *n* = 2) from the control group. These animals were transported to the laboratory, and euthanised by an overdose of isoflurane, followed by cervical dislocation, and immediately frozen at -80°C until dissection (see '4*.3.3 Analyses of gut samples*' below).

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 antibiotic or a sham control, 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 presented in Chapter 3 (see '*3.3.3 Analyses of gut samples*', see also 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 the stomach, small intestine, caecum, and colon. The luminal contents and membrane of each section was scanned for helminths at 10× magnification (Leica© MS5 microscope with a Leica© CLS100 light attachment). Faeces were homogenised in TBS and scanned for helminths at 10× magnification. Helminths were collected and pooled according to species, gut section and mouse individual in 70% ethanol for future size and fecundity analyses (see '*4.3.9 Helminth size and fecundity measurements*'). A bacterial pellet was obtained from the gut and faecal material using the following centrifugation steps: total contents of the tube were centrifuged for 950 G for 10 minutes at 4°C, resulting in a pellet containing the gut membrane and non-bacterial lumen contents (e.g., digested food). This pellet was discarded, but the supernatant was further centrifuged at 9000 G for 15 minutes at 4°C. The resulting supernatant was discarded and the remaining bacterial pellet was immediately stored at -80°C for future bacterial DNA analysis (see '4*.3.4 16S rRNA gene sequencing*' below).

4.3.4 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 (Cavedine *n* = 9, Pietramurata *n* = 5 individuals) from the antibiotic group, and 19

samples from 8 individuals from the control group (Cavedine n = 1, Pietramurata n = 7 individuals). In addition, small intestine, caecum and colon samples from the 26 euthanised individuals were sequenced. Preparation of samples (DNA extraction, DNA quantification, PCR and PCR product purification), and subsequent sequencing of the resulting amplicon library followed methods presented in Chapter 3 (see '*3.3.4 16S rRNA gene sequencing*').

4.3.5 Bioinformatic processing of 16S data

Sequences were merged, trimmed and filtered using MICCA software (version 1.5.0, Albanese *et al.*, 2015) following methods provided in Chapter 3 (see '*3.3.5 Bioinformatic processing of 16S data*').

4.3.6 Statistical analyses of microbiota - diversity

Generalised linear mixed models (GLMMs) were used to assess whether there was an association between microbiota alpha diversity and antibiotic treatment, using the inverse Simpson index. 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 random

intercepts for each model. Statistical analyses used the package *glmmADMB*, version 8.3.3 (Fournier *et al.*, 2012; Skaug *et al.*, 2016). A process of multi-model inference was used to compare all possible models using the R package *MuMIn* (Bartoń, 2015), and the most parsimonious model was selected using a threshold of ΔAICc <2 (Burnham and Anderson, 2003).

4.3.7 Statistical analyses of microbiota - composition

A distance-based redundancy analysis (db-RDA; capscale function in R package *vegan*; Oksanen *et al.*, 2017) 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 (see '*3.3.8 Statistical analyses of microbiota – composition*' in Chapter 3 for more details). 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.

4.3.8 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 in *DESeq2*, and significance values (p <0.05) were derived using likelihood-ratio tests (Anders and Huber, 2010; Love *et al.*, 2014).

4.3.9 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, in preparation for morphological analyses. 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 helminth area was calculated. 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.

4.3.10 Statistical analyses of helminth abundance, EPG, 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 of helminth egg shedding (eggs per gram of faeces; 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 helminth 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 random intercepts for each model. Statistical analyses used the package *glmmADMB*, version 8.3.3 (Fournier *et al.*, 2012; Skaug *et al.*, 2016). For each GLMM, a process of multi-model inference was used to compare all possible models using the R package *MuMIn* (Bartoń, 2015). The most parsimonious model was selected using a threshold of ΔAICc <2 (Burnham and Anderson, 2003).

4.4 Results

4.4.1 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. Antibiotic treatment did not affect gut microbiota alpha diversity; there was no significant difference in inverse Simpson indices for microbiota of the small intestine (d.f. = 7, $Z = 1.89$, $p = 0.06$), caecum (d.f. = 7, $Z = -0.98$, $p = 0.33$), colon (d.f. = 8, $Z = -1.14$, $p = 0.25$) or

faeces (d.f. = 30, Z = -1.60, p = 0.11) between pre- and post-treatment individuals. In the control group there were also no significant differences in microbiota alpha diversity of the caecum (d.f. = 7, Z = 0.77, p = 0.44), colon (d.f. = 10, Z = -0.06, p = 0.96), or faeces (d.f. = 14, Z = 0.22, p = 0.82) between pre- and post-treatment individuals (Figure 4.1). However, in the control group the microbiota alpha diversity of the small intestine was significantly higher in post- compared to pre-treatment individuals (d.f. = 10, Z = 2.71, p <0.01; Figure 4.1).

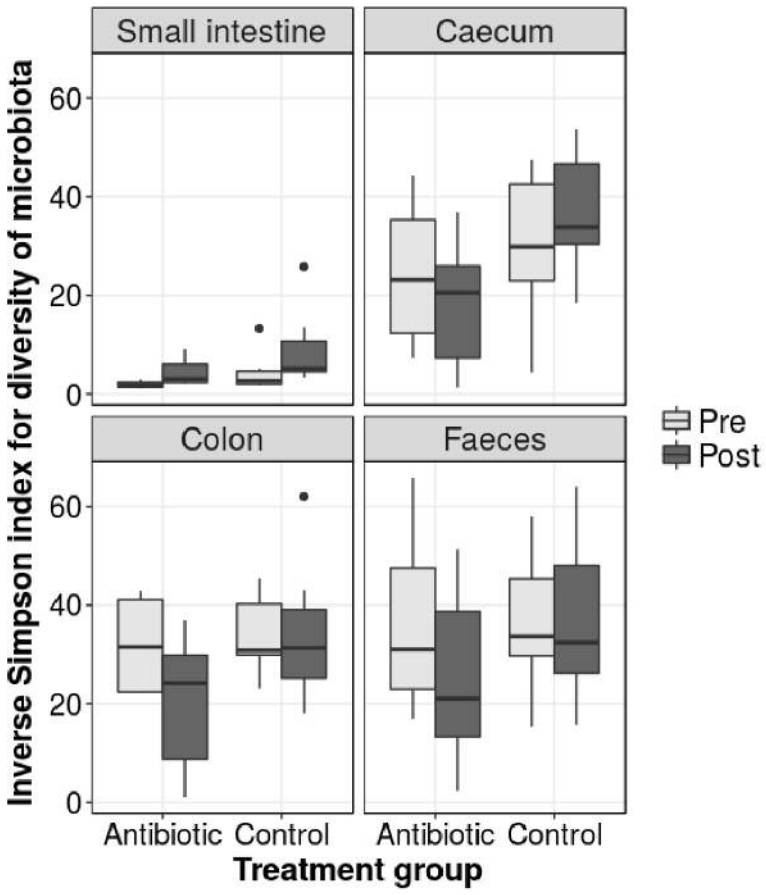

Figure 4.1: Inverse Simpsons diversity index for microbiota in different gut sections and faeces of pre- and post-treatment mice in an antibiotic or control group. Boxes demonstrate the upper and lower quartiles of alpha diversity, with median alpha diversity indicated. Bars represent the minimum and maximum range of alpha diversity.

4.4.2 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 4.2). 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 4.2).

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 4.2, 4.3, 4.4 and 4.5). 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 4.3), caecum (Bray-Curtis: d.f. = 20, F = 1.46, p = 0.01; weighted UniFrac: d.f. = 20, F = 2.7, p = 0.02; Figure 4.4), 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 4.4). Faeces partially followed this pattern; faecal 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 4.5).

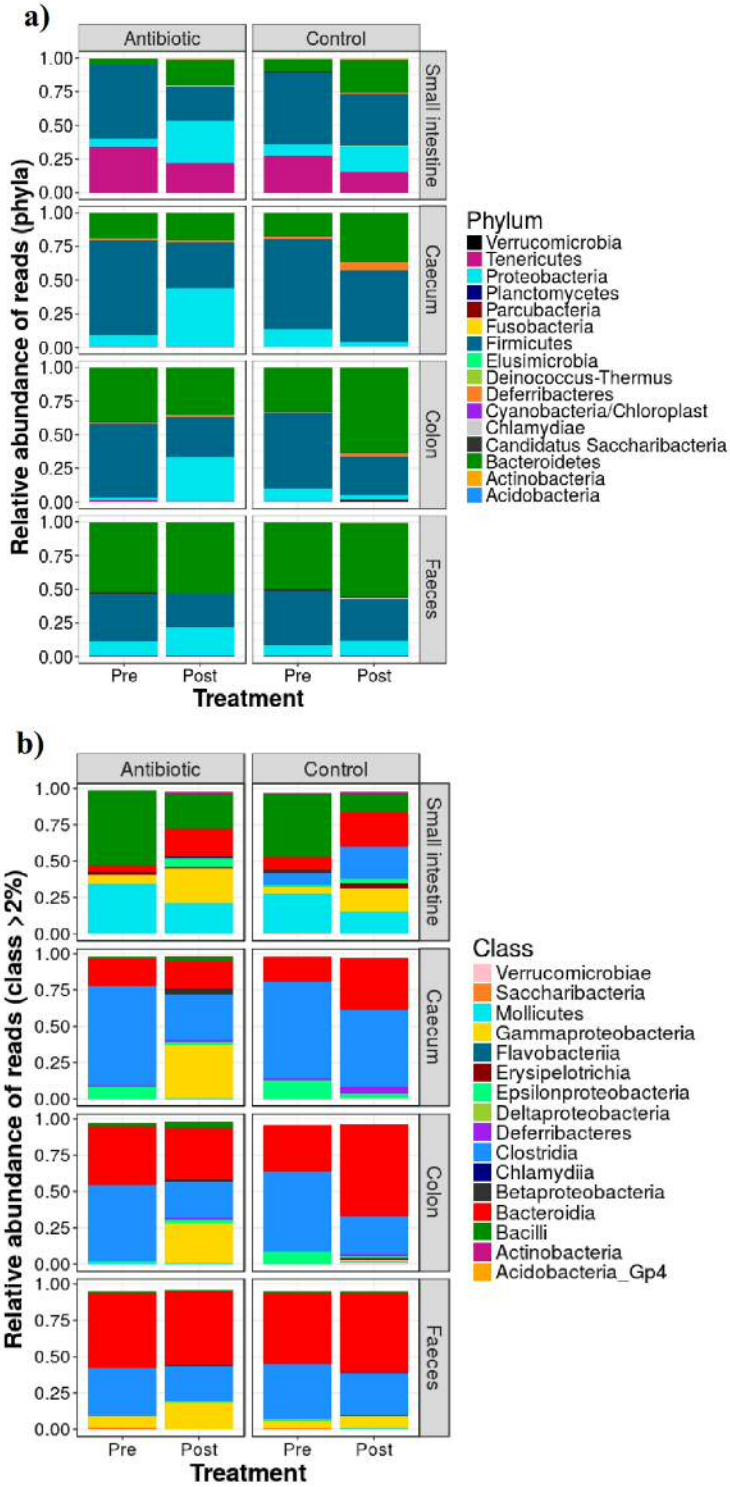

Figure 4.2: Relative abundance of reads of bacterial a) phyla and b) classes (>2%) present in different gut sections and faeces of mice pre- and post-treatment with antibiotic or a control sham gavage.

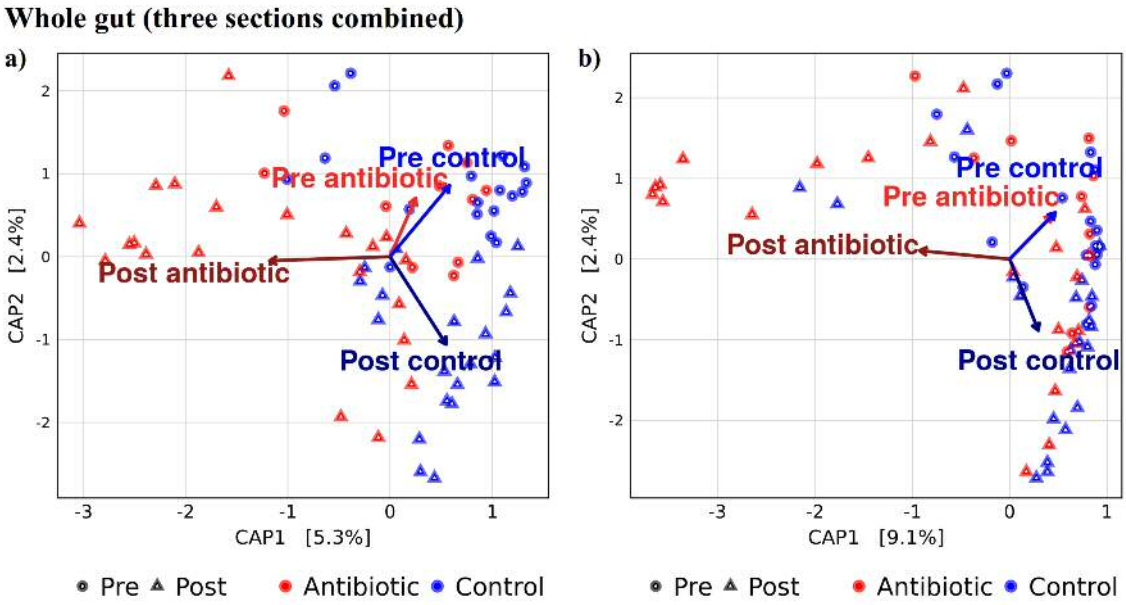

Figure 4.3: Ordination plots of divergence of microbiota taxonomic composition between samples of three gut sections (small intestine, caecum and colon) combined, associated with treatment with either antibiotic or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. 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.

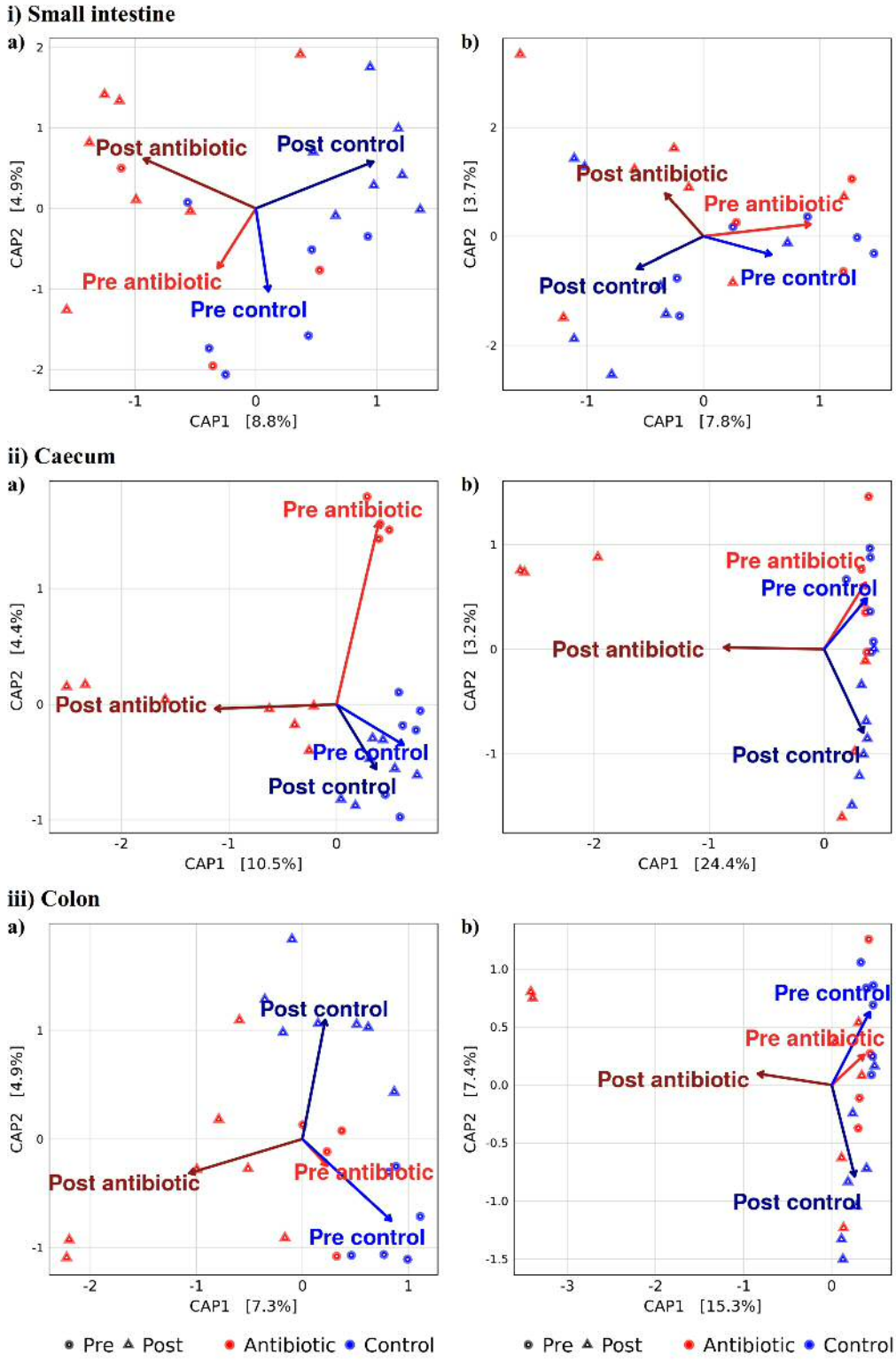

Figure 4.4: Ordination plots of divergence of microbiota taxonomic composition between i) small intestine, ii) caecum and iii) colon samples, associated with treatment with either antibiotic or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples 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.

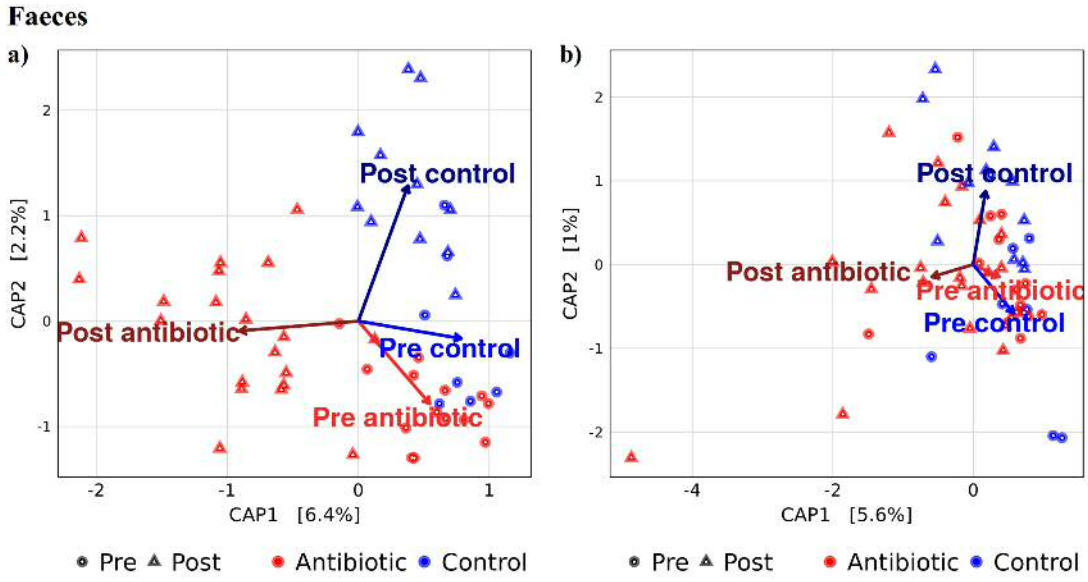

Figure 4.5: Ordination plots of divergence of microbiota taxonomic composition between faecal samples associated with treatment with either antibiotic or a control sham gavage, based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. Distribution of samples along the first two db-RDA axes (i.e., CAP1 and CAP2) and associated proportion of variation are shown. The length of the arrows indicate the relative importance of each treatment.

4.4.3 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 4.6; 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 4.6). 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 abundance decreased. Bacteria in four

other phyla exhibited changes in abundance between pre- and post-treatment individuals in both the antibiotic and control group (Figure 4.6; Appendix A.4).

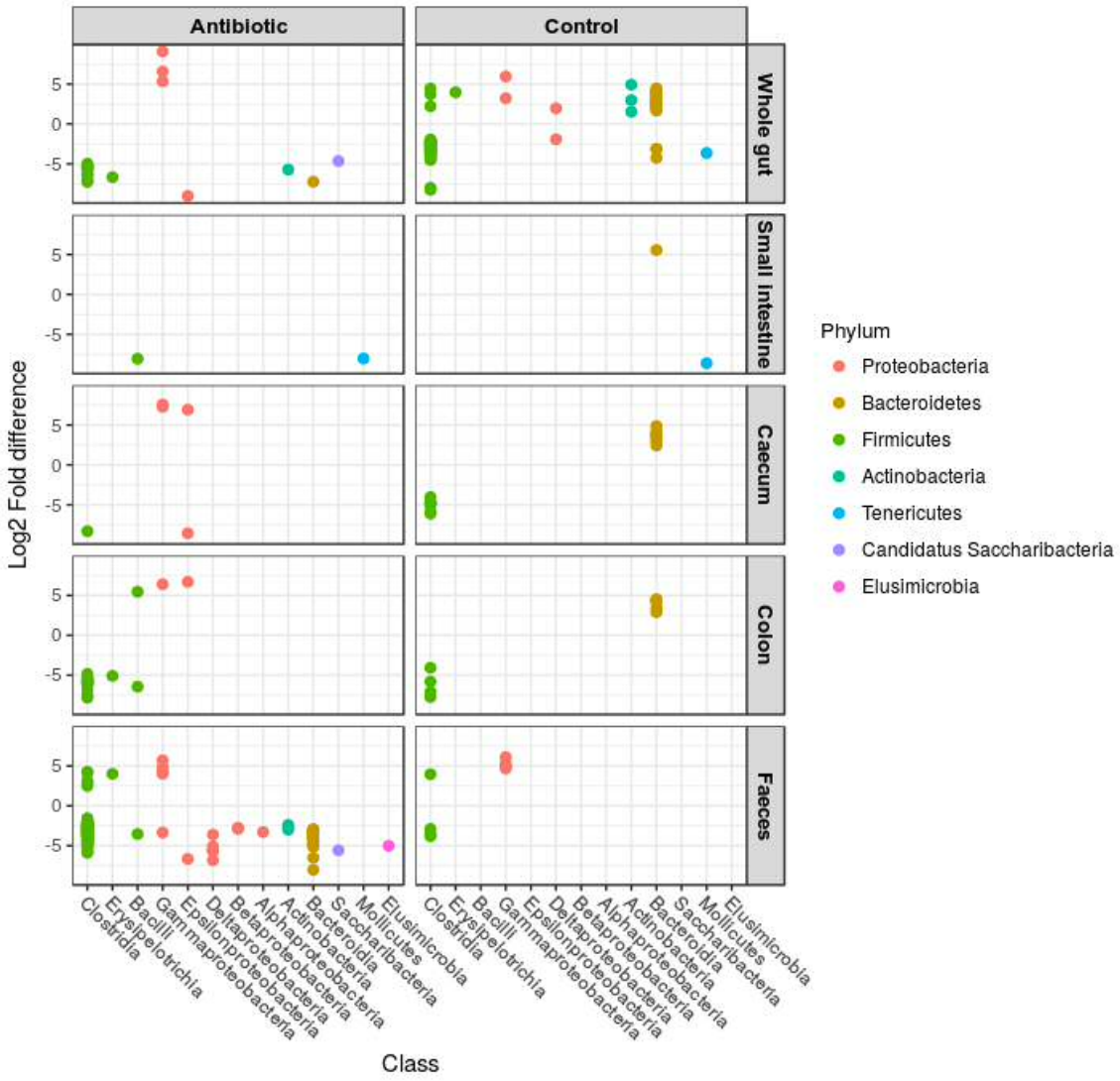

Figure 4.6: OTUs in the gut microbiota that were significantly different in abundance in post-treatment compared to pre-treatment individuals in an antibiotic treatment and control group. Microbiota of the whole gut (three gut sections combined), small intestine, caecum, colon and faeces were analysed. 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.

4.4.4 The effect of antibiotic treatment on helminth prevalence and abundance

Helminths from the species *A. tetraptera*, C. *vitta*, *H. polygyrus*, *Hymenolepis* spp., *M. muris*, *S. frederici* and *T. muris* were isolated from the guts of mice, however the prevalences and/or abundances of *A. tetraptera*, *C. vitta*, *M. muris*, *S. frederici* and *T. muris* were insufficient for individual analyses, and were instead included in 'all helminth' analyses (Table 4.1, see Appendix A.4, Figure A.4.1 for boxplots of analysed helminth abundance data). 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 4.7). 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 was not associated with significant changes in total helminth abundance (d.f. = 9, Z = -1.07, p = 0.28), nor the abundances of either *H. polygyrus* (d.f. = 9, Z = 0.18, p = 0.85) or *Hymenolepis* spp. (d.f. = 9, Z = -1.05, p = 0.30; Table 4.1; Figure 4.7). Likewise, there were no significant differences in total helminth (d.f. = 10, Z = -0.64, p = 0.52), *H. polygyrus* (d.f. = 8, Z = -0.84, p = 0.40), or *Hymenolepis* spp. abundances (d.f. = 9, Z = 0.70, p = 0.49) between pre- and post-treatment individuals in the control group (Table 4.1, Figure 4.7).

Table 4.1: Mean abundance (± standard error of mean) of helminths isolated from the gut of pre- or post-treatment individuals in an antibiotic or control group.

Helminth species	Helminth abundance			
	Antibiotic		Control	
	Pre	Post	Pre	Post
Total	55.5 ± 23.6	30.3 ± 7.4	75.3 ± 43.9	25.1 ± 10.7
A. tetraptera	0.2 ± 0.2	0.1 ± 0.1	0	0
C. vitta	0.7 ± 0.7	0	0	0
H. polygyrus	23.8 ± 17.5	27.3 ± 6.5	11.7 ± 5.0	7.3 ± 1.6
Hymenolepis spp.	20.5 ± 16.9	2.7 ± 1.5	10.2 ± 4.8	17.9 ± 11.2
M. muris	6.7 ± 6.7	0	0	0
S. frederici	3.3 ± 3.3	0	53.5 ± 41.0	0
T. muris	0.3 ± 0.3	0.1 ± 0.1	0	0

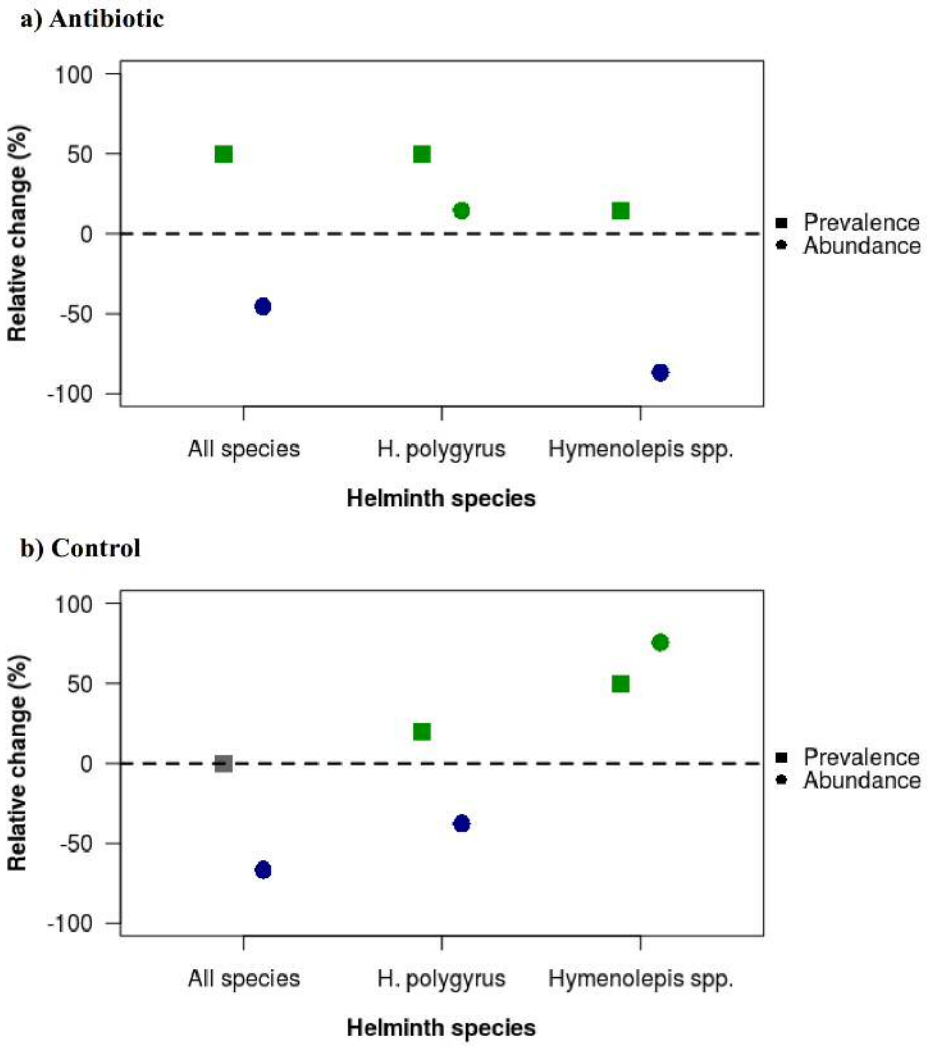

Figure 4.7: Relative changes (%) in helminth prevalence and abundance between pre- and post-treatment individuals in an a) antibiotic and b) control group for all helminth species, *Heligmosomoides polygyrus* and *Hymenolepis* spp. Prevalence and abundance of other identified species were insufficient 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.

4.4.5 The effect of antibiotic treatment on helminth egg shedding

Eggs from *H. polygyrus*, *Hymenolepis* spp. and *T. muris* were identified in mouse faeces, however the number of *T. muris* eggs were insufficient for individual analyses and were instead included in 'all helminth' analyses (Table 4.2; see Appendix A.4, Figure A.4.2 for boxplots of analysed helminth EPG data). Antibiotic treatment was linked to consistent and substantial increases in

helminth egg shedding (eggs per gram of faeces; EPG); mean EPG increased by 790.1% from pre- to post-treatment in the antibiotic group (d.f. = 75, Z = 2.58, p = 0.01), *H. polygyrus* EPG increased by 362.4% (d.f. = 75, Z = 2.66, p <0.01), while *Hymenolepis* spp. EPG increased by 2,164.7% (d.f. = 74, Z = 2.24, p = 0.03; Figure 4.8; Table 4.2). In the control group there was no significant change in total egg shedding (d.f. = 51, Z = 0.75, p = 0.45), nor in *H. polygyrus* (d.f. = 51, Z = -0.55, p = 0.58) and *Hymenolepis* spp. egg shedding (d.f. = 51, Z = 0.58, p = 0.56) between pre- and post-treatment individuals (Figure 4.8; Table 4.2).

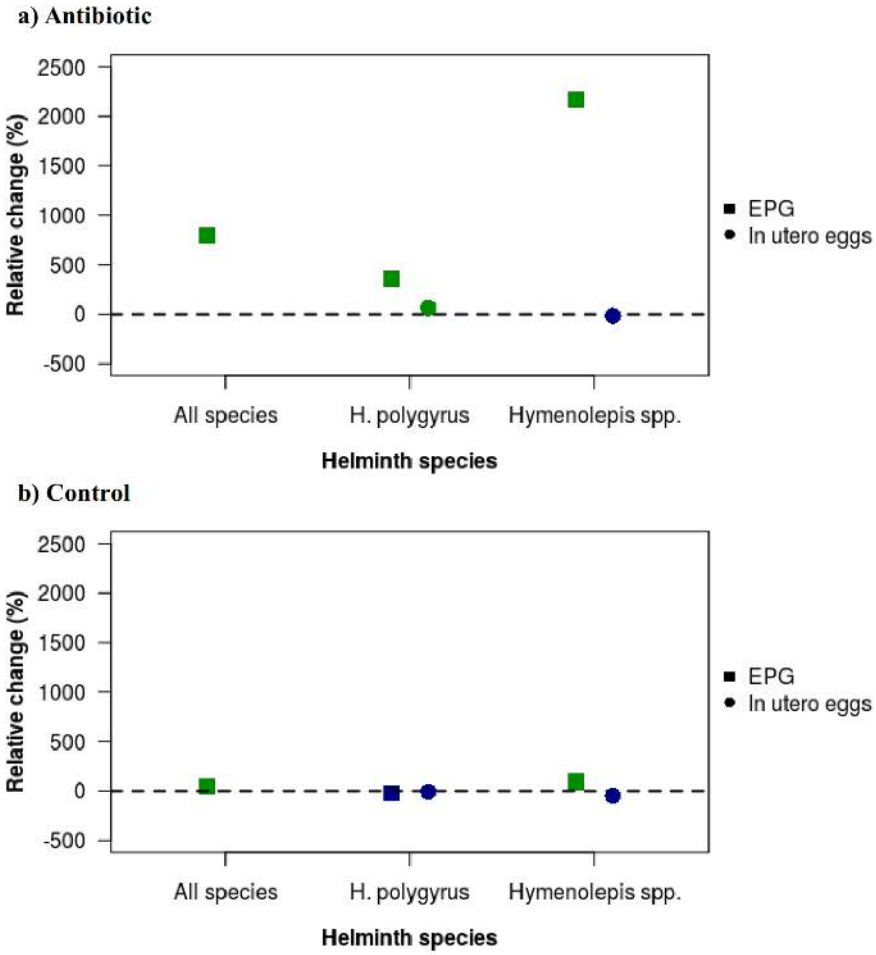

Figure 4.8: Relative changes (%) in eggs per gram (EPG) of faeces and *in utero* egg abundance between pre- and post-treatment individuals in an a) antibiotic and b) control group for all helminth species, *Heligmosomoides polygyrus* and *Hymenolepis* spp. Prevalence and abundance other identified species 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.

Table 4.2: Mean number of helminth eggs per gram (EPG) of faeces (± standard error of mean) in faecal samples collected from pre- or post-treatment individuals in an antibiotic or control group.

Helminth species	Helminth EPG			
	Antibiotic		Control	
	Pre	Post	Pre	Post
Total	154.5 ± 46.7	1,375.4 ± 289.6^{+}	546.5 ± 223.2	814.3 ± 246.8
H. polygyrus	117.0 ± 38.3	545.0 ± 120.1^{+}	207.6 ± 98.3	172.0 ± 68.4
Hymenolepis spp.	36.7 ± 31.7	830.4 ± 276.7^{+}	317.8 ± 212.6	622.1 ± 252.2
S. frederici	0	0	0	0
T. muris	0	0	21.2 ± 21.2	17.7 ± 13.0

$^{+}$ *Represents a significant increase in EPG between pre- and post-treatment individuals in either an antibiotic or control group.*

4.4.6 The effect of antibiotic treatment on helminth fecundity, percentage of females and size

In utero egg counts were performed for *H. polygyrus* and *Hymenolepis* spp. The prevalences and abundances of *A. tetraptera*, *C. vitta*, *M. muris*, *S. frederici* and *T. muris* were insufficient for individual fecundity analyses (Table 4.3, see Appendix A.4, Figure A.4.3 for boxplots of analysed helminth fecundity data). Antibiotic treatment had no significant impact on the fecundity (number of *in utero* eggs/μm^2 of helminth) nor the percentage of females of *H. polygyrus* (d.f. = 93, Z = 1.00, p = 0.32 and d.f. = 7, Z = 0.63, p = 0.53 respectively; Figure 4.8; Table 4.3). *Hymenolepis* spp. also did not exhibit any significant changes in fecundity associated with antibiotic treatment (d.f. = 40, Z = -0.65, p = 0.52; Table 4.3). Although there was no significant difference in helminth size between pre- and post-antibiotic treatment for *H. polygyrus* (d.f. = 209, Z = 0.39, p = 0.70), *Hymenolepis* were 229.5% larger in post-treatment individuals (d.f. = 44, Z = 4.06, p <0.01; Table 4.3). In the control group there was no significant difference between pre- and post-treatment individuals in fecundity, percentage of females or size of *H. polygyrus* (d.f. = 48, Z =

-0.43, $p = 0.67$; d.f. = 9, $Z = 0.38$; and $p = 0.70$; d.f. = 105, $Z = -0.17$, $p = 0.86$ respectively), nor the fecundity or size of *Hymenolepis* spp. (Figure 4.8; Table 4.3).

Table 4.3: Mean fecundity, measured as number of *in utero* eggs/μm^2 of helminth (± standard error of mean) of helminths collected from pre- or post-treatment individuals in an antibiotic or control group.

Helminth species	Helminth fecundity (eggs/μm^2)				Helminth females (%)				Helminth size (μm^2)			
	Antibiotic		Control		Antibiotic		Control		Antibiotic		Control	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
H. polygyrus	23.6 ± 3.7	37.9 ± 9.2	16.5 ± 3.0	32.9 ± 3.6	50.9	51.7	64.6	54.7	0.62 ± 0.04	0.71 ± 0.06	0.85 ± 0.08	0.80 ± 0.06
Hymenolepis spp.	220.5 ± 25.7	57.7 ± 13.1	151.4 ± 16.2	12.6 ± 1.6	NA	NA	NA	NA	9.3 ± 2.7	57.7 ± 13.1$^+$	19.2 ± 2.0	12.6 ± 1.6

NA = not applicable (helminth species is hermaphrodite).

$^+$ *Represents a significant increase in a parameter between pre- and post-treatment individuals in either an antibiotic or control group.*

4.5 Discussion

The present study demonstrates that antibiotic treatment of the host is associated with significant increase in helminth egg shedding, and a significant increase in the size of *Hymenolepis* spp. 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 (790.1% for all helminth species, 362.4% for *H. polygyrus* eggs and 2,164.7% for *Hymenolepis* eggs) and significant. However, *in utero* egg counts were not significantly different between pre- and post-antibiotic treatment individuals, suggesting that the absolute (but not net) rate of egg production within helminths increased, to match the higher rate of egg shedding, such that the number of *in utero* eggs remained constant.

It should be noted that egg shedding data (faecal egg counts) were collected over time for each individual, however due to the destructive nature of sampling adult helminths within the gut, it was possible to perform *in utero* egg counts only at a single time point for each mouse individual, which may have differed from the time point that egg shedding data were collected. Consequently, it is not possible to accurately 'match' the *in utero* fecundity and egg shedding data, not least because they were collected from different individuals (not all individuals were euthanised due to ethical reasons, e.g., pregnant females were not euthanised). Thus, it is also possible that antibiotic treatment of the host stimulated helminths to simultaneously shed all eggs, which were 'replaced' by newly produced eggs by the time *in utero* egg counts were performed. Unfortunately, it is not possible to distinguish from the collected data if the increase in egg shedding associated with antibiotic treatment was persistent or increased as a 'pulse' after treatment; although trapping was performed frequently and consistently animals were not always re-captured at regular time

intervals (as discussed in more detail in Chapter 3). Consequently, data collected post-treatment ranged from 1 – 29 days from the first date of treatment of an individual, such that little can be reliably ascertained (statistically) about the temporal pattern since time of treatment of EPG increases following antibiotic treatment.

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 of antibiotic 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 the 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 shed significantly more eggs in faeces (Figure 4.8). In addition, Bacilli in the small intestine showed a significant decrease in abundance (Figure 4.6). Instead, in the control group Bacilli abundance did not significantly change, and there were no changes in *H. polygyrus* prevalence, abundance, size or fecundity in the control group (Figure 4.7 and 4.8). 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). Results in the present study also elude to a positive association between *Hymenolepis* spp. and Clostridia; *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 (non-significant) 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 and shed significantly more eggs (Dezfuli *et al.*, 2002; Lagrue and Poulin, 2008).

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. However, it is not possible to determine from the data collected in the current study if bacterial abundances decreased after antibiotic treatment. While the administered cocktail of antibiotics was originally designed to obtain gnotobiotic (individuals with defined/depleted or absent microbiota) mice in the sterile environment of the laboratory by twice daily administration (Reikvam *et al.*, 2011), due to ethical and practical restrictions the individuals in the current study were treated every seven days, between which time they were exposed to the bacteria-rich environment of the field sites. It is therefore very unlikely that microbiota of mice in the current study were depleted to the extent achieved in the laboratory study (Reikvam *et al.*, 2011), and quantitative PCR would be necessary to confirm this, as 16S rRNA Illumina sequencing does not currently provide accurate quantitative data (e.g., see Kennedy *et al.*, 2014). We do know, however, that diversity of microbiota did not significantly

change between pre- and post-antibiotic treatment individuals, but composition of microbiota did in all gut sections except the small intestine. Thus, changes in gut microbiota composition associated with antibiotic treatment were either driven by changes in the abundances of bacteria OTUs already present in the gut, or there was no significant net change in the loss and gain of different bacteria OTUs.

Antibiotic may also have affected helminths by indirectly affecting crucial bacterial symbionts within the host gut. For example, some helminth species may rely on bacteria in the host gut 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, which may have been affected by antibiotic treatment of the host. Evidence has shown that both free-living and parasitic nematodes can harbour a microbiota (Tan and Grewal, 2001; Lacharme-Lora *et al.*, 2009a, 2009b; 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 within the host following treatment with the antibiotics administered in the present study (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 subsequently be 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, antibiotic treatment is associated with increases in helminth prevalence. Increased prevalence of helminths following antibiotic treatment may be a knock-on effect associated with an increase in helminth egg shedding 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 all have incited changes in the helminth community (Hayes *et al.*, 2010; Reikvam *et al.*, 2011; Biswal *et al.*, 2016). The work presented here suggests that antibiotic can lead to increased helminth egg shedding into the environment, leading to higher rates of transmission in the host population.

4.6 Author Acknowledgements

The manuscript resulting from this chapter is authored by:

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E.L.P: Designed study, collected, analysed and interpreted data, drafted the manuscript.

J.K: Involved in designing study, provided guidance regarding appropriate analyses for microbiota data.

M.C: Provided guidance regarding processing of microbiota samples/data collection.

H.C.H: Involved in conceiving the study and organising field work and logistics, provided comments on each version of the manuscript.

J.R.M: Provided comments on the manuscript.

S.E.P: Conceived and designed study, involved in data interpretation, provided comments on each version of the manuscript.

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

A burgeoning research area focusses on the importance of, and interactions between, microbiota and parasitic helminths in the gut. However, as yet unconsidered, are the helminth-associated microbiota; which could affect helminth and even host health. Here we describe, for the first time, the diversity and taxonomic composition of microbiota associated with six parasitic helminth species from naturally infected wild rodents, and the gut niche in which they were co-located. Helminth microbiota exhibited both intra- and interspecific variation. *Heligmosomoides polygyrus* were associated with the most taxonomically rich microbiota: 257 different genera were identified across all sequenced sample. However, mean alpha diversity was highest in *T. muris* (33.0 ± 4.3 standard error). At the other extreme, samples from three helminth species were associated with a single OTU that constituted ≥99% microbiota, including putatively pathogenic bacteria genera: 50.5% of *Hymenolepis diminuta* samples, 12.5% *Aonchotheca murissylvatici*, and a single *M. muris*. For all helminth species except *H. diminuta*, intraspecific microbiota variation was driven by gut location of the helminth. In addition, for all but one species (*Mastophorus muris*), alpha diversity of the helminth microbiota exceeded that of its gut niche for at least one sequenced sample, and the taxonomic composition of helminth microbiota was significantly different to that of the gut, e.g., *Deferribacteres* constituted 38.0% bacterial reads from *S. frederici*, but only 1.0% reads from all gut sections. Thus, community assembly of helminth-associated microbiota may occur and/or bacteria are derived from non-host sources, e.g., an intermediate host or during free-living stages in the environment. These data provide the first steps to identifying microbes associated with helminths that are potentially crucial for helminth survival.

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 rapidly expanding area of research (Chapter 2). The gut microbiota in particular has so many important functions within the host that it has earned the accolade 'the undiscovered organ' (Bäckhed *et al.*, 2005). The parasitic helminths are frequent and abundant in the gut, and ubiquitous across species, 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). Recent work has shown a clear interaction between the gut microbiota and helminths, for example, bacterial diversity within the host gut often increases following helminth infection (Walk *et al.*, 2010; Rausch *et al.*, 2013; Cantacessi *et al.*, 2014; Lee *et al.*, 2014; Kreisinger *et al.*, 2015). Interestingly, the discovery of parasite-microbiota interactions has led to further discussion on the positive health benefits of parasites, resulting from the helminth-driven changes in bacterial diversity and composition (Walk *et al.*, 2010; Broadhurst *et al.*, 2012; Giacomin *et al.*, 2016b). While there is uncertainty surrounding the mechanisms by which helminths may modulate host microbiota, a number of theories have been proposed; parasite secretions may have antimicrobial properties, damage to the gut epithelium resulting from parasite attachment may alter the gut environment and therefore the ability of certain bacterial species to proliferate, and/or microbial changes may be mediated by parasite-microbiota immune interplay (Glendinning *et al.*, 2014; Reynolds *et al.*, 2014; Giacomin *et al.*, 2016a).

The microbiota of parasitic species is currently an area of research interest; mainly on the microbial communities associated with 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 the importance of these parasites as pathogen vectors. In addition, previous work has shown that both parasitic and non-parasitic helminths can be associated with bacteria, such as *Wolbachia* (Taylor *et al.*, 2005; Lacharme-Lora *et al.*, 2009; Plieskatt *et al.*, 2013; Berg *et al.*, 2016; Derycke *et al.*, 2016), and some helminths may be able to vector bacteria to the host which is either pathogenic to the host in its own right (Tan and Grewal, 2001; see also Perkins and Fenton, 2006), or may contribute to the pathogenesis of the helminth infection (Saint André *et al.*, 2002; Brattig, 2004). However, very little research has been dedicated to characterising the entire microbial community associated with parasitic helminth species (Walk *et al.*, 2010; Plieskatt *et al.*, 2013). 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; indeed, some antibiotics have already been shown to reduce fitness, slow development and inhibit motility of helminths (Wells, 1951; Brown, 1952; Wells, 1952a, 1952b; Salem and el-Allaf, 1969; Hoerauf *et al.*, 1999; Saint André *et al.*, 2002; but see also Chapter 4).

Some bacteria are known to be important to helminth 'health', as is the case with *Wolbachia* spp., which, within helminths, appears to be found strictly within some 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 and imaging have identified possible ectosymbionts (Bakke *et al.*, 2006) and bacteria in the lumen (Cable and Tinsley, 1991) of different monogenean species, bacteria associated with the tegument of cestodes (Poddubnaya and Izvekova, 2005), as well as vertically transmitted micro-organisms within helminth tissue, e.g., the hypodermis, which in some cases may adversely affect helminth

development (Anderson *et al.*, 1973; Mclaren *et al.*, 1975; Kozek and Marroquin, 1977; Franz and Büttner, 1983; see also Bakke *et al.*, 2006; Morley, 2016 for reviews). Pathogenic bacteria have also been observed in helminths, although largely within free-living non-parasitic nematodes, and only rarely in parasitic species (Mclaren *et al.*, 1975; Kozek and Marroquin, 1977; Franz and Büttner, 1983; Perkins and Fenton, 2006), but this lack of evidence may well be due to a lack of observations. Collectively, these studies certainly suggest that bacteria are associated with helminths. However, there are few studies that have examined or indeed characterised a larger microbial community associated with parasitic helminths.

Although few in number, studies have thus far provided positive evidence for a helminth microbiota composed of multiple bacteria species. Cultured livestock nematodes are associated with a bacterial community, possibly acquired from the host faeces in which the helminths develop (Lacharme-Lora *et al.*, 2009). Notably, Lacharme-Lora *et al.*, (2009) utilised culture-dependent techniques, which are unlikely to have identified the full consortia of bacteria associated with the parasites, since not all bacteria in microbiota can be cultured (Suau *et al.*, 1999). More recently, culture-independent techniques have shown that non-parasitic nematodes (*Caenorhabditis elegans*) harbour a consistent 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 overall *C. elegans* microbiota composition (Berg *et al.*, 2016; Dirksen *et al.*, 2016). Until now just two studies have described microbiota of parasitic helminths using culture-independent techniques; laboratory strains of the small intestinal nematode of mice; *H. polygyrus bakeri* (Walk *et al.*, 2010), and the liver fluke *Opisthorchis viverrini,* which can infect humans (Plieskatt *et al.*, 2013). However, as observed in other taxa it is possible that the microbiota of laboratory-derived helminths is reduced in diversity in comparison

with wild 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. Given that studies using microscopy, and both culture-dependent and -independent methods have found initial evidence of a helminth microbiota, it is timely that helminth-associated microbiota should be investigated 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 the helminths were isolated is described, in order to ask the questions 'what is the microbiota composition and diversity of a helminth community?', 'is there intraspecific variation of helminth microbiota between gut locations?' and 'is the helminth microbiota unique, or similar to that of the host?'

5.3 Materials and Methods

5.3.1 Sample collection

Thirty-two adult *Apodemus flavicollis* (14 females and 18 males) were live-trapped from April to July 2015 in mature beech forests (*Fagus sylvatica* L.) with understorey 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. Faeces were collected from traps occupied by a single individual. 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. Faeces collected from traps from each individual were homogenised in TBS and scanned for parasitic helminths at 10× magnification. Helminths from a single individual were collected in TBS according to species and the gut location from which the helminths 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 were manually removed using sterile tweezers. External debris was further removed from each sample of helminths by transferring helminths to a 20 µm pore cell strainer and washing with 50 ml of fresh 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 gut contents and bacteria were collected with the rest of the luminal contents in a centrifugation tube. A bacterial pellet was obtained from faecal and gut samples 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 mouse individual from which the helminths 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 sequenced sample (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 methods provided by the manufacturer for the isolation of genomic DNA from tissue, with the addition of carrier RNA. Recovered DNA was quantified using a Qubit 2.0 Fluorometer with a Qubit® dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified using the primers 341F and 805R (see Appendix A.2, Figure A.2.1 for details on primer sequences, including degenerate nucleotides). 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. Quantification, purification and normalisation of

the PCR products, plus subsequent sequencing of the resulting amplicon library followed methods presented in Chapter 3 (see '*3.3.4 16S rRNA gene sequencing*').

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. Primers were trimmed from the resulting merged 16S rRNA fragments, which were then discarded if they had an average expected error (AvgEE) probability 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 – diversity and composition

The inverse Simpson index was used to calculate alpha diversity of OTUs in each gut section and helminth batch. Spearman's rank correlation coefficient was used to test if alpha diversity of a helminth batch was correlated with the number of individual helminths within a batch, for each species. Distance-based redundancy analyses (db-RDA; capscale function in R package *vegan*) were used to test if intraspecific variation in microbiota composition observed between batches within a given helminth species was associated with presence within different gut locations. In

addition, db-RDA analyses were used to test for taxonomical differences in microbiota composition between a given gut section (each sequenced stomach, small intestine, caecum, proximal colon, distal colon or faecal sample, regardless if gut section was infected by helminths) and batches of helminth species therein. No helminths were isolated from within faeces, but helminth and faecal microbiota comparison analyses were included, as faeces may provide a source of helminth-associated bacteria. 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 – BLAST comparison with soil microbiota

To determine if helminths acquired bacteria from the soil during free-living life stages, helminth microbiota sequences were compared to those of soil using the BLAST (Basic Local Alignment Search Tool) algorithm. A search in Web of Science® was performed to find publically available soil microbiota sequences from similar habitat to that of the study area. Search terms were ‘microbi*’ AND ‘soil’ AND ‘alp*’ OR ‘Italy’ OR ‘beech’ OR ‘forest’. The titles of resulting articles were scanned for relevance and Rasche *et al.*, (2011) was considered to contain the most relevant data; soil microbiota from a temperate beech forest in Austria. The publically available soil microbiota sequences were downloaded (from NCBI PopSet: 300807846) and concatenated into a single fasta file with which to provide a reference. The sequences from each helminth

species were also concatenated into single fasta files, from which nucleotide databases were produced. These databases containing helminth microbiota sequences were BLASTed against those from the soil microbiota, using BLAST software (Altschul *et al.*, 1990). Significance was based on an *E* value threshold of $E < 0.05$ and bitscore of 300.

5.3.6 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 *DESeq2* package, version 1.14.1 (Anders and Huber, 2010; 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 of helminth infected every mouse 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 most (19,258 ± 13,692), but the number of reads from *T. muris* remained most consistent (18,022 ± 6,764; Table 5.1).

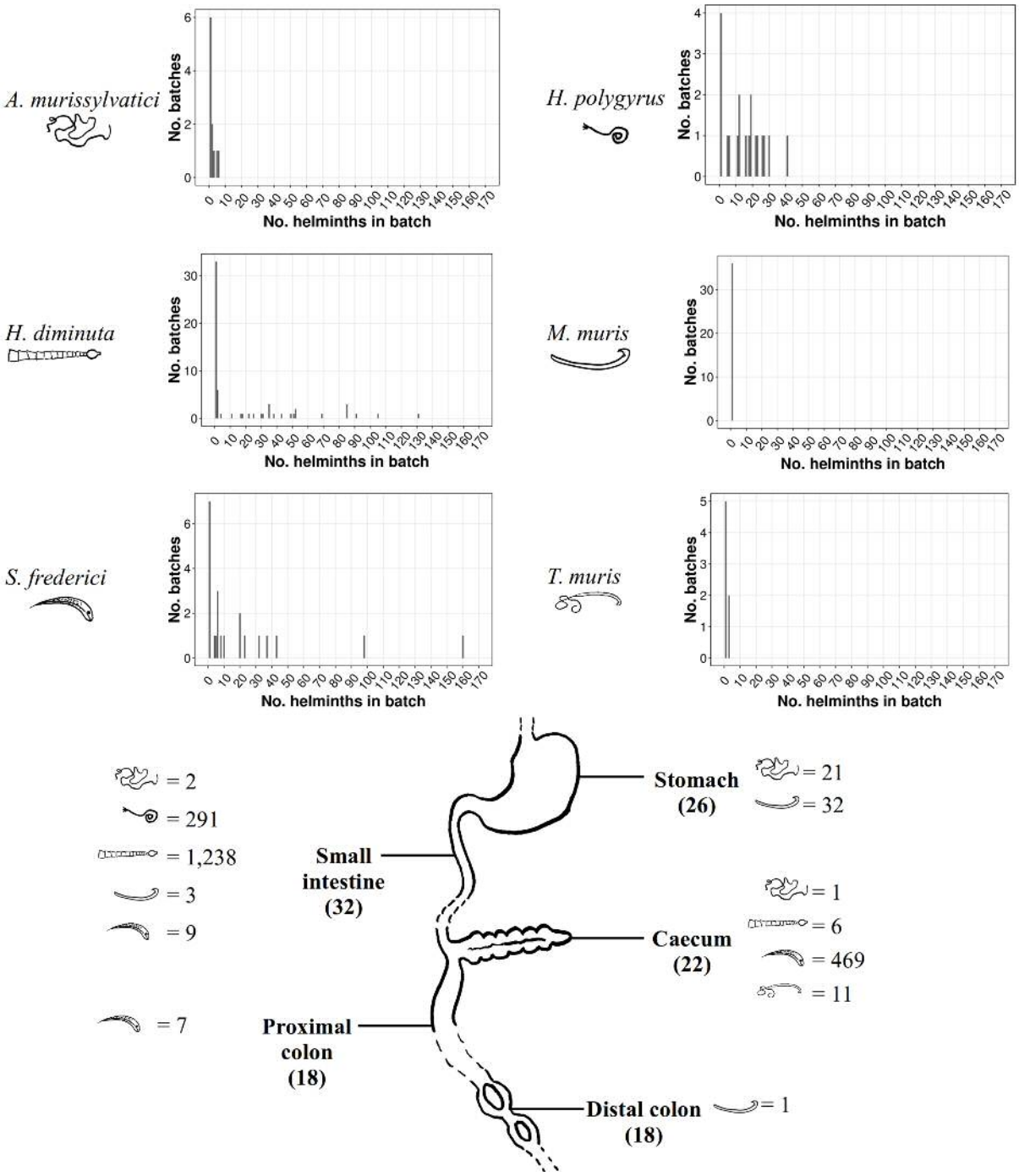

Figure 5.1: Helminth and gut samples that were sequenced from 32 *Apodemus flavicollis* individuals for analysis of microbiota diversity and composition. Microbiota were analysed from six helminth species; *Aonchotheca murissylvatici, Heligmosomoides polygyrus, Hymenolepis diminuta, Mastophorus muris, 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 phylum 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 or 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

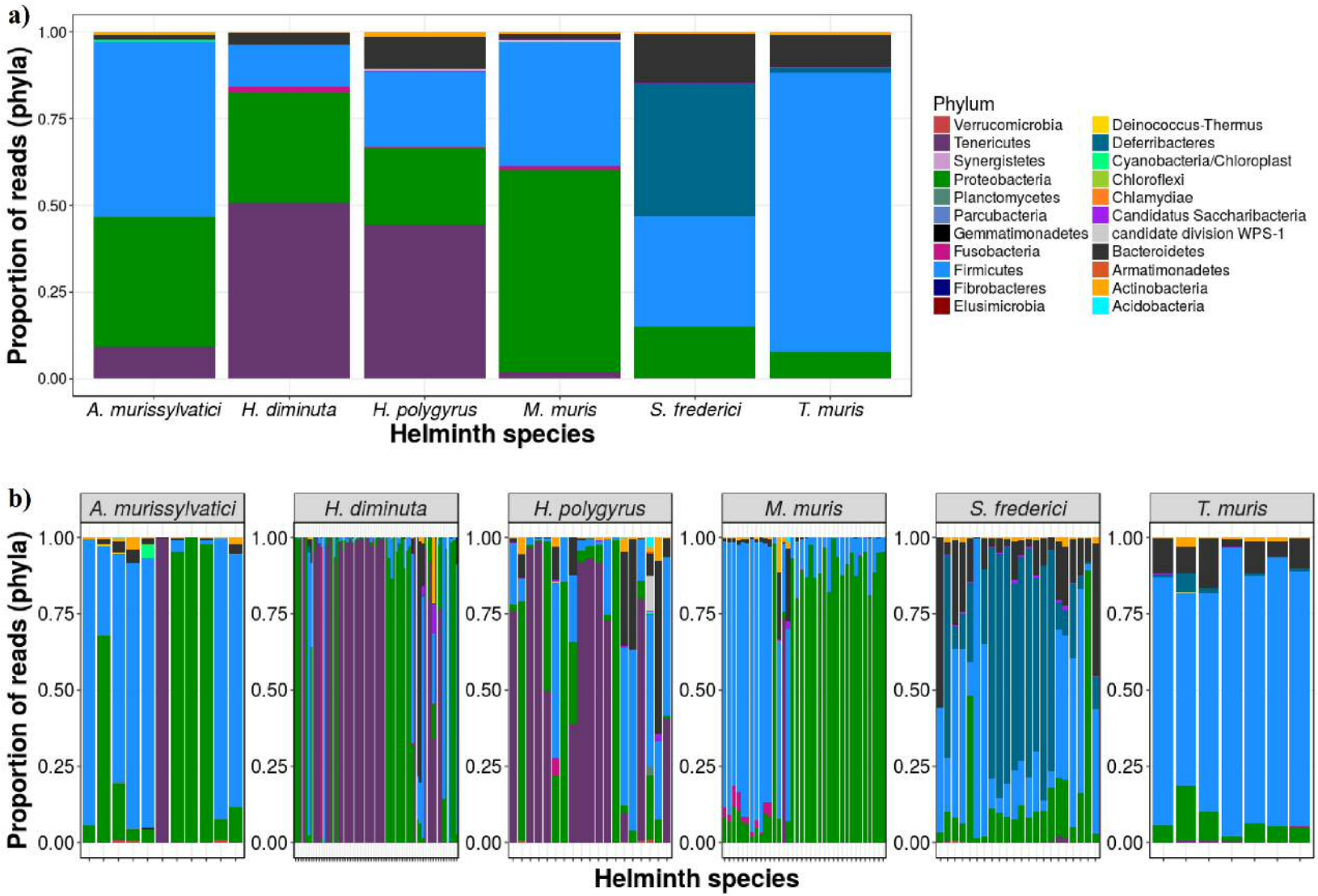

Figure 5.2: Proportion of reads composed of different bacterial phyla in microbiota associated with a) six helminth species and b) individual samples composed of either a single helminth or pooled individuals (according to gut location and individual from which the helminths were isolated) for each of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

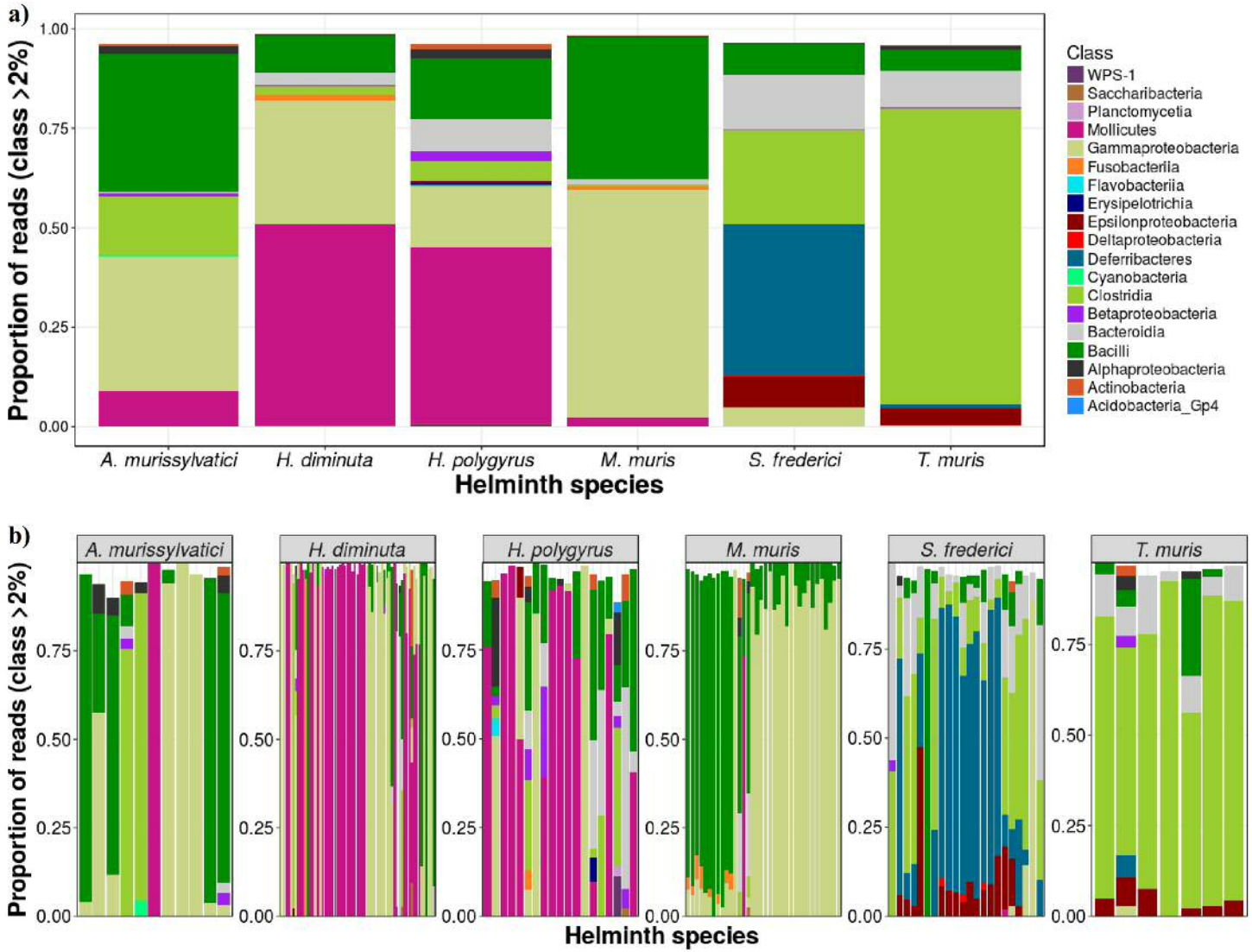

Figure 5.3: Proportion of reads (composing >2% of the total) of different bacterial phyla in microbiota associated with a) six helminth species and b) individual samples composed of either a single helminth or pooled individuals (according to gut location and individual from which the helminths were 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 sample of *S. frederici* (n = 160 helminths in sample), 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, $Z = -2.14$, $p = 0.03$).

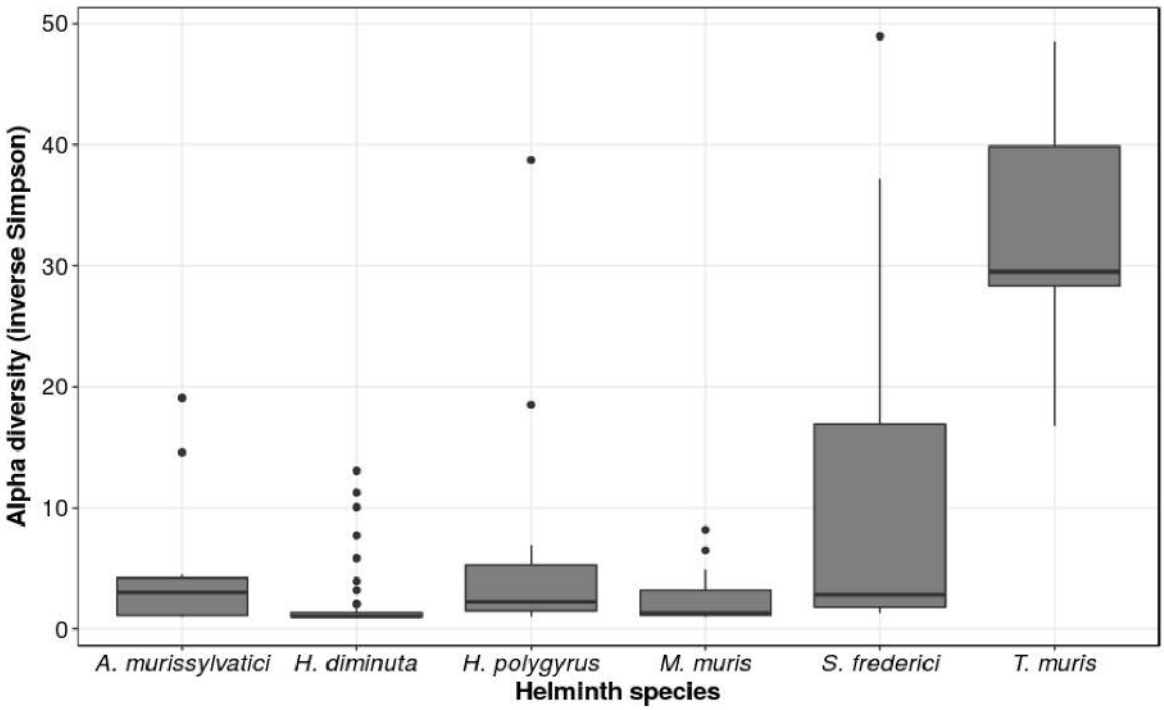

Figure 5.4: Inverse Simpson index of alpha diversity of microbiota from six helminth species isolated from the guts of 32 *Apodemus flavicollis*. Boxes demonstrate the upper and lower quartiles, with median alpha diversity indicated. Bars represent the minimum and maximum range of alpha diversity.

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 sections. Intraspecific variation in taxonomic composition of microbiota associated with a given helminth species 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 microbiota with gut and soil 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 the helminth 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 sequenced 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 host 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 host caecum in which the helminths were present (d.f. = 12, $W = 62$, $p = 0.5$; Figure 5.5). Following BLAST analyses, 28.6% (8/28) of the bacterial classes identified in *A. murissylvatici* were present in soil microbiota, followed by 25.0% (7/28) in *H. diminuta*, 24.1% (7/29) in *S. frederici*, 23.1% (6/26) in *M. muris* and 15.8% (6/38) in *H. polygyrus*. There were no classes of bacteria from soil microbiota that were significantly present in *T. muris.*

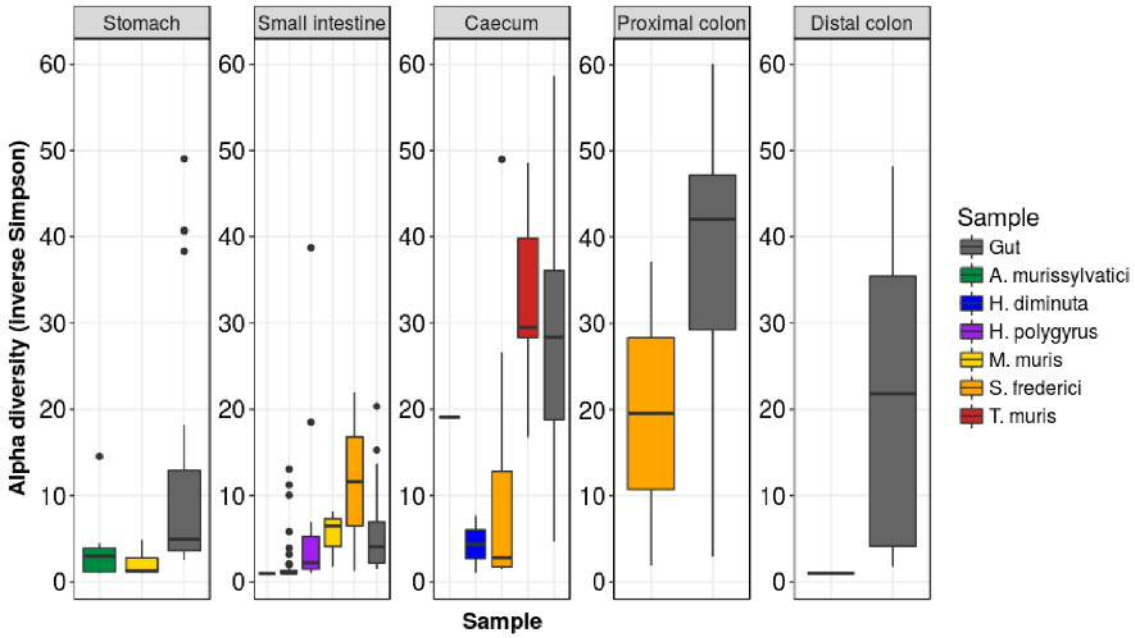

Figure 5.5: Inverse Simpson index of alpha diversity of microbiota associated with different gut locations, as well as alpha diversity of microbiota associated with six helminths species isolated from each respective gut location. Boxes demonstrate the upper and lower quartiles, with median alpha diversity 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 (thus were more similar) 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, $F = 5.8$, $p<0.01$). There were no helminths isolated from within faeces collected from occupied traps, and helminth microbiota was significantly different to that of the faecal microbiota (Bray-Curtis: d.f. = 163, $F = 8.83$, $p<0.01$; weighted UniFrac: d.f. = 163, $F = 13.5$, $p<0.01$; Figure 5.6)

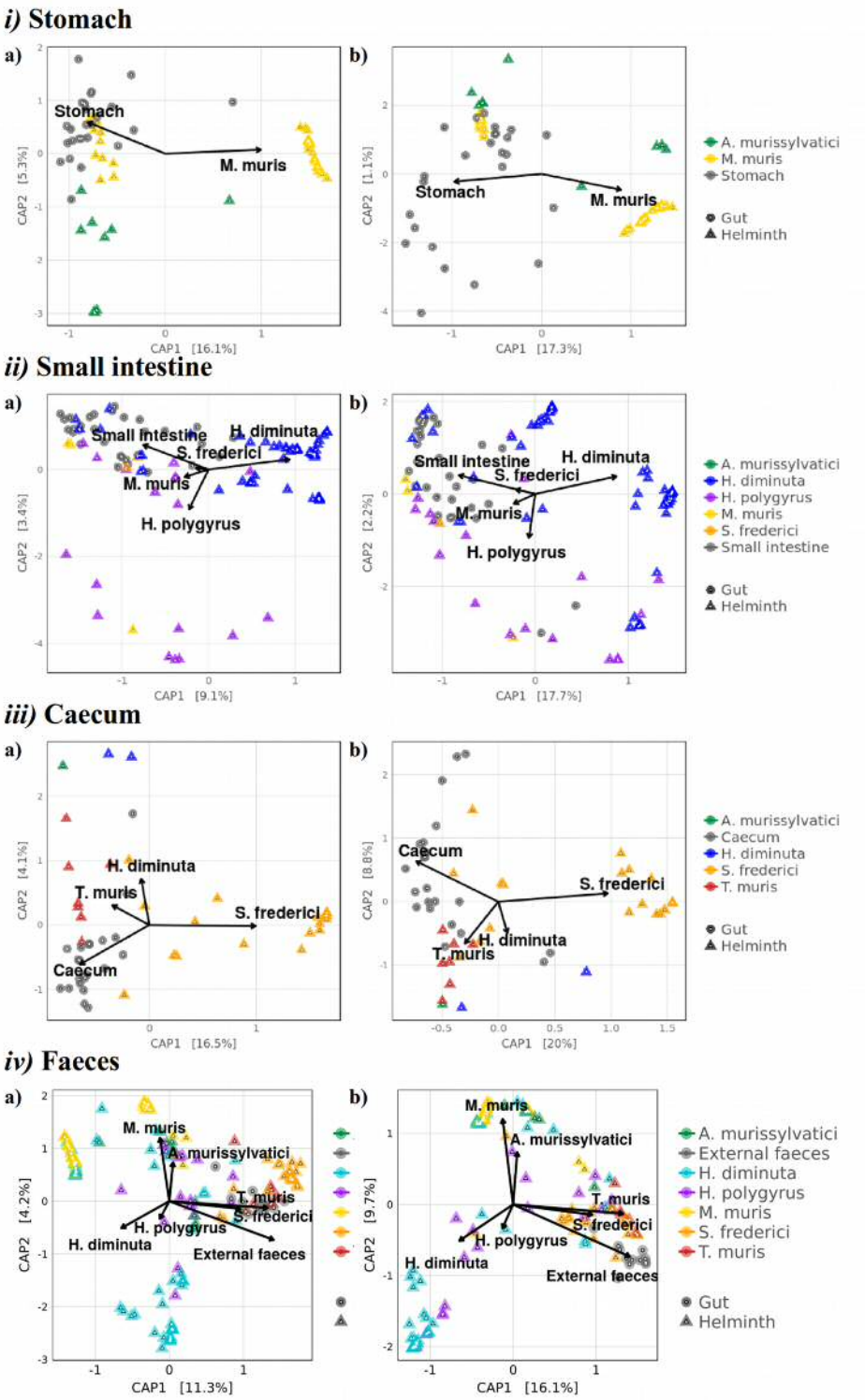

Figure 5.6: Ordination plots of divergence of microbiota taxonomic composition between the i) stomach, ii) small intestine iii) caecum or iv) faeces, and that of the helminth species therein based on a) Bray–Curtis and b) weighted UniFrac dissimilarities. In the case of faeces, all helminth samples were included within this analysis despite the fact no helminths were present within faeces. 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).

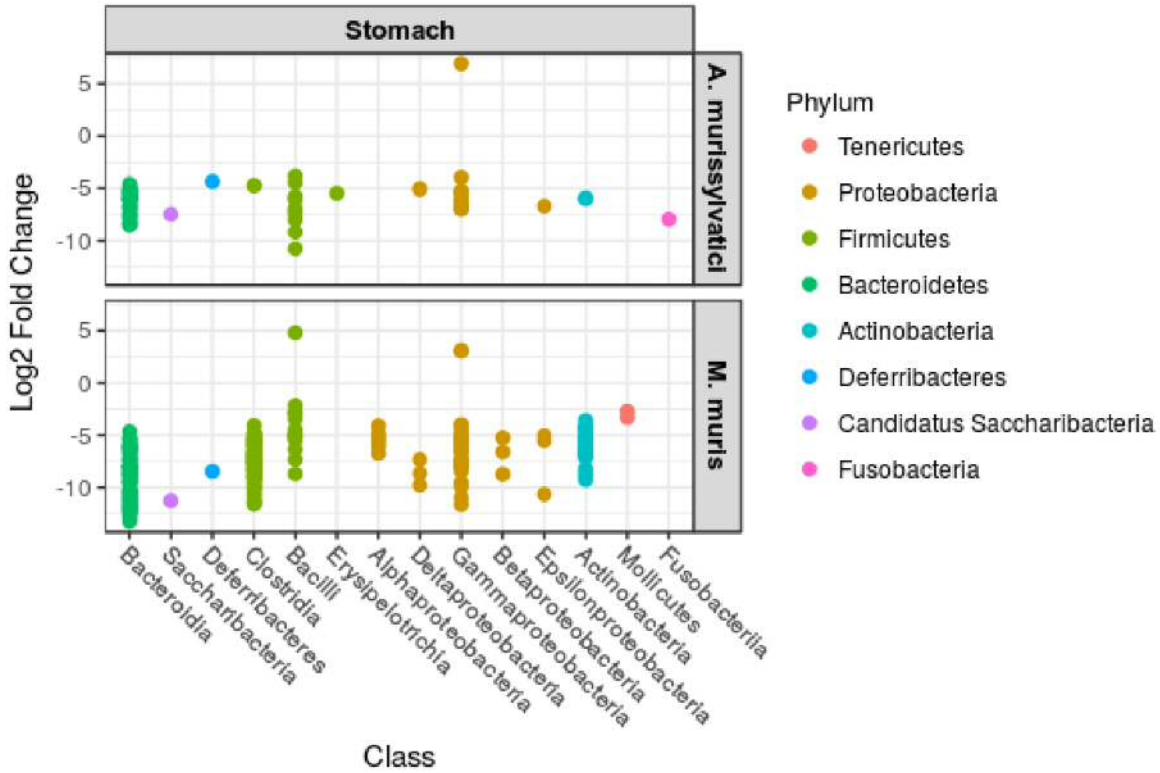

Figure 5.7: OTUs in the microbiota of helminths isolated from the stomach (*Aonchotheca murissylvatici* and *Mastophorus muris*) that were significantly different to those present in the stomach microbiota. OTUs were grouped by microbial class. Briefly, DESeq was used to identify significantly changing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

Mollicutes were 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 7 phyla (7 classes) 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).

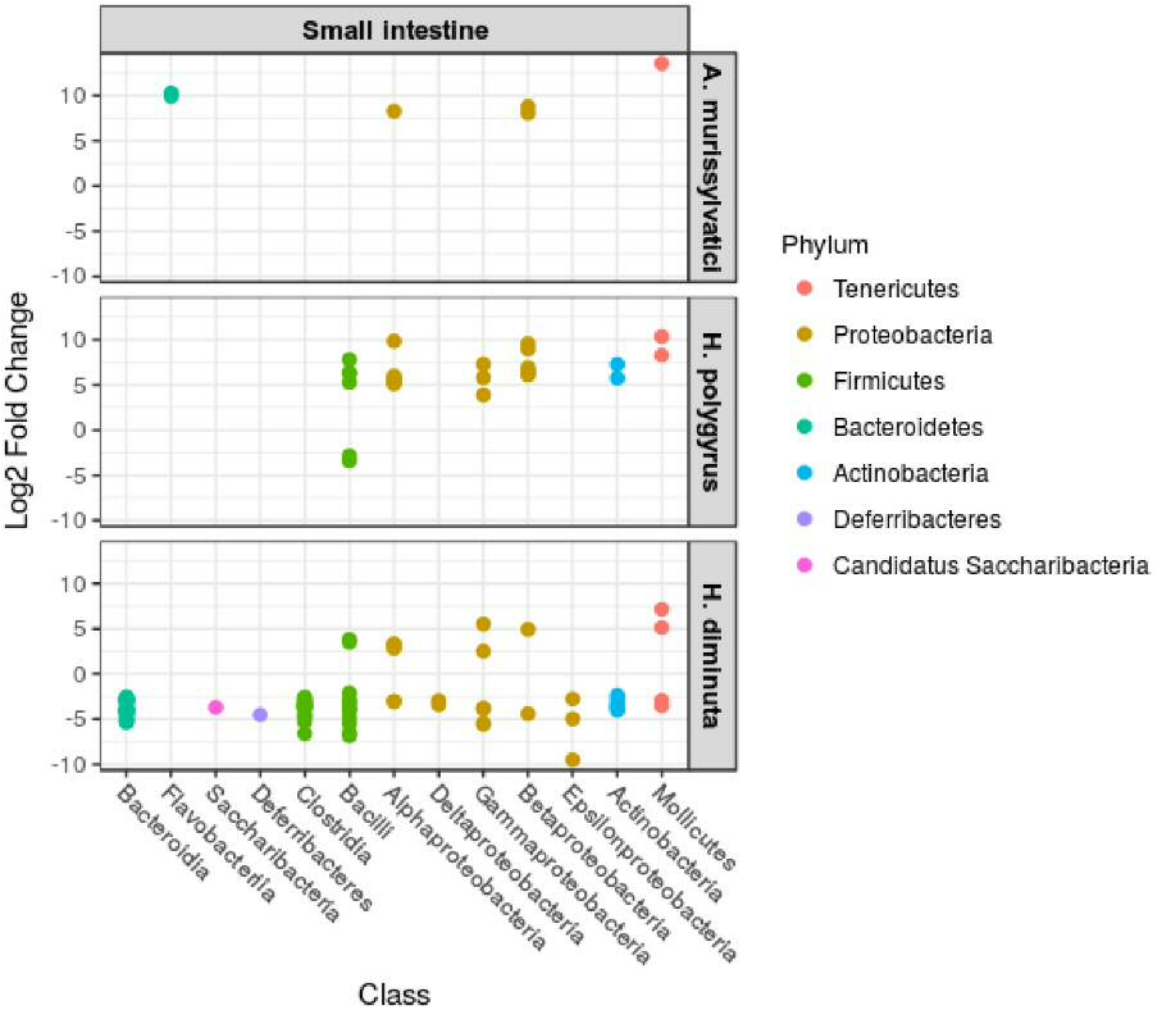

Figure 5.8: OTUs in the microbiota of helminths isolated from the small intestine (*Aonchotheca murissylvatici, Heligmosomoides polygyrus* and *Hymenolepis diminuta*) that were significantly different to those present in the small intestine microbiota. OTUs were grouped by microbial class. Briefly, DESeq 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 6 phyla (10 classes) were significantly higher in abundance, and OTUs from 7 phyla (10 classes) 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).

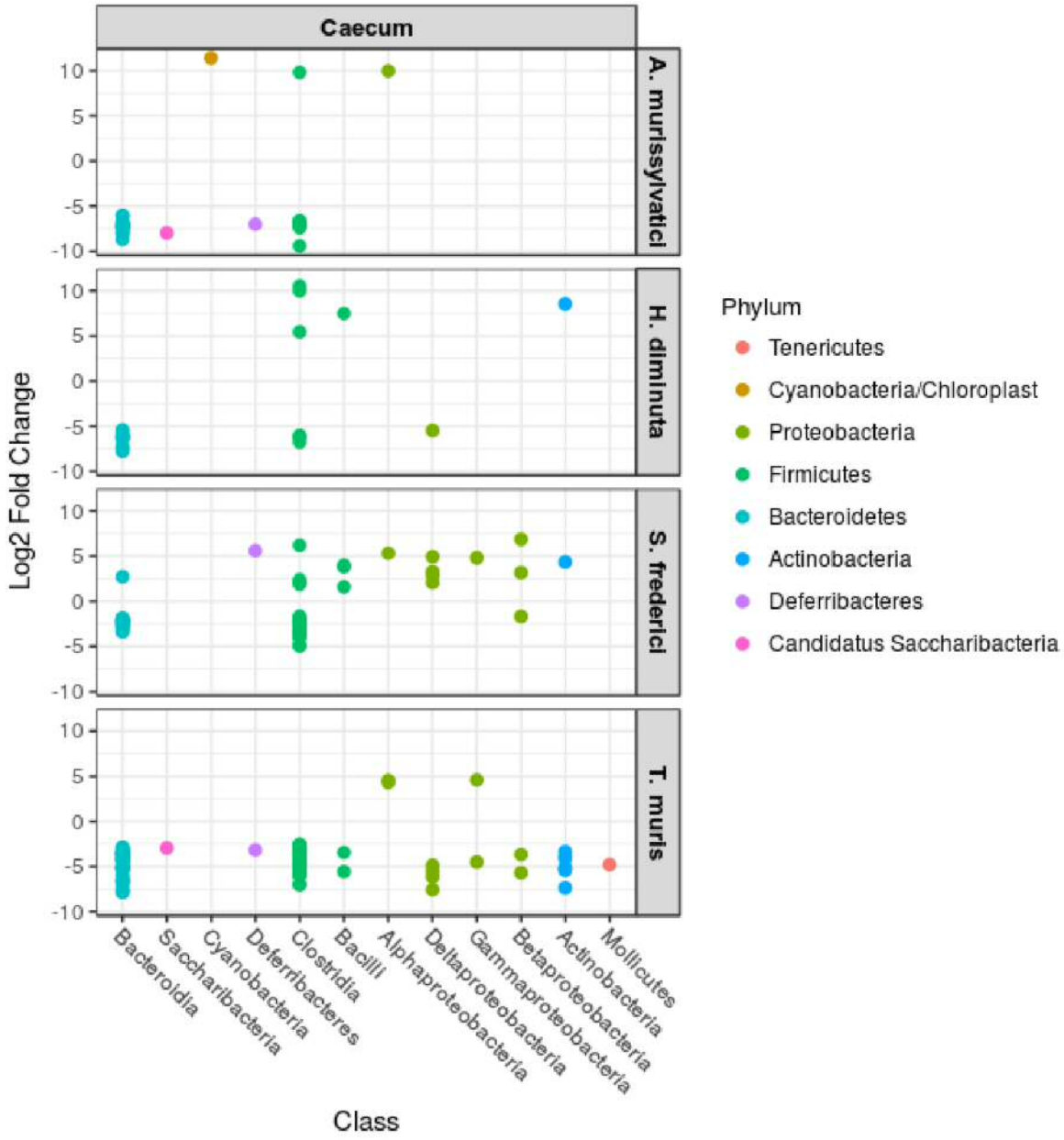

Figure 5.9: OTUs in the microbiota of helminths isolated from the caecum (*Aonchotheca murissylvatici, Hymenolepis diminuta, Syphacia frederici* and *Trichuris muris*) that were significantly different to those present in the caecum microbiota. OTUs were grouped by microbial class. Briefly, DESeq was 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 *S. frederici* 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).

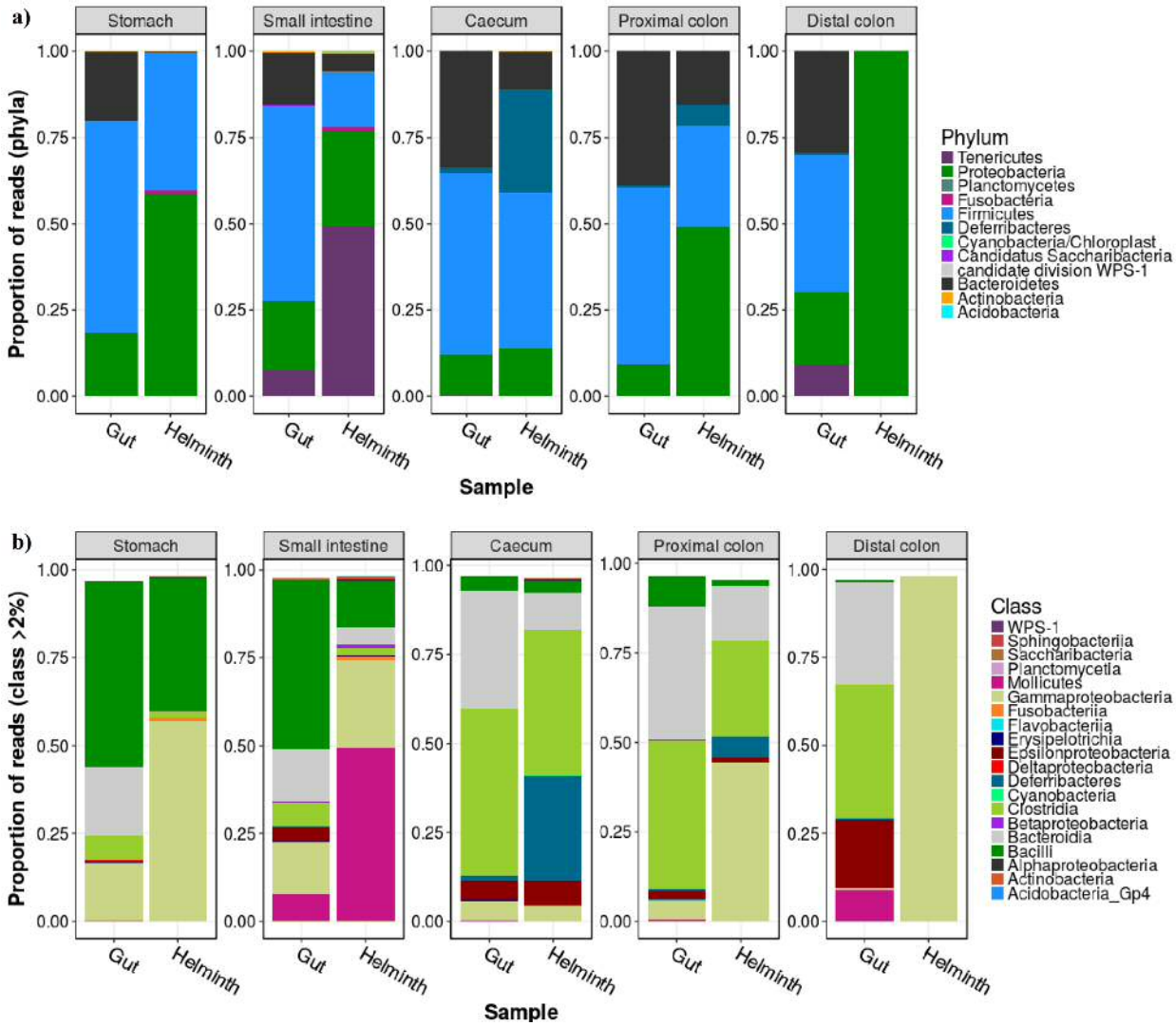

Figure 5.10: Proportion of reads of bacterial a) phyla and b) classes (composing >2% reads) for gut and all helminth samples located within five gut locations. Gut and helminth samples were collected from 32 *Apodemus flavicollis*.

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 number of 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 bacteria from the genera Mycoplasma, Escherichia/Shigella or Lactobacillus. Likewise, *A. murissylvatici* samples had a monoculture microbiota of Mycoplasma, and a single *M. muris* had a microbiota composed of a Escherichia/Shigella monoculture. 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 can even kill the *C. 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 or metagenomics would be necessary to confirm that the OTUs observed here were indeed pathogenic species (at least to mammals).

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. It is possible that *H. diminuta* microbiota does not differ between gut sections as cestodes are typically composed of a tegument with very few internal organ 'niches' that could be colonised by different bacteria at different host gut sections. Although *A. murissylvatici* significantly formed different taxonomic clusters 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 impacted the helminth microbiota composition. Indeed, the *M. muris* from the distal colon was amongst those to harbour a monoculture bacteria, suggesting that a single genera of bacteria had proliferated within the helminth, perhaps because the helminth could no longer modulate microbiota due to death.

In general, helminth microbiota had lower alpha diversity than the microbiota of the gut section from within which the helminth 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 modulated by the helminth 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 the helminth species was found, in at least one sequenced sample, suggesting that microbiota were acquired from sources other than the host gut (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 are parasitic to 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, larvae may still harbour microbes acquired 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* 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.

The current study aimed to test if helminth microbiota may have been acquired from faeces or soil during the free-living stages of the helminth. Microbiota associated with faecal samples was significantly different to microbiota associated with all helminth species, thus it is unlikely that a significant amount of bacteria associated with helminths is acquired from faeces. However, faecal microbiota were obtained from faeces that had accumulated overnight in occupied traps, over which time the faecal microbiota may have changed and become contaminated (e.g., with concentrated levels of mouse urine associated with being in a confined space, etc.), thus may not have provided an accurate representation of the faecal microbiota to which helminths in the free-living stage are exposed to. Comparison of soil and helminth microbiota was achieved by BLASTing helminth microbiota sequences against those from soil microbiota in a beech forest

(similar habitat in which mice were captured). There were bacteria classes present in the helminth microbiota that were also present in soil for all helminth species except *T. muris*, and for some species (*A. murissylvatici* and *H. diminuta*) more than a quarter of bacterial classes present in the helminth microbiota were also identified in soil. While this does not provide direct evidence that helminths acquire microbiota from the soil, it does suggest that future experiments on the helminth microbiota should also sequence soil samples from the site of study for more reliable comparisons of helminth and soil microbiota; as the beech forest soil microbiota study (Rasche *et al.*, 2011) used very different methods to those used in the current chapter (e.g., qPCR analyses, annotation of sequences based on the NCBI as opposed to RDP database), this very much limited the analyses that could be performed on these data. Results should therefore be cautiously interpreted, particularly as soil microbiota can vary significantly between areas even of similar habitat (Lazzaro *et al.*, 2015).

In the current study excess bacteria on the external surface of helminths was removed by multiple TBS washing steps, however, bacteria originating from the host gut would undoubtedly have remained on helminths. It is very difficult to tease apart whether the bacteria that remained on helminths following external washing are part of the helminth associated microbiota, or are primarily associated with the host gut and are simply passively present on the helminth. Despite the high chance that these external host-acquired 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 the helminth was 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 from 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).

There is overwhelming evidence that parasitic helminths possess a microbiota, however the current study characterised bacteria associated with the entire helminth, and did not take into consideration which tissues/organs of the helminth these microbes were associated with. Previous microscopy and imaging studies have identified bacteria in the gut lumen, body surface/tegument, reproductive apparatus and glandular cells of helminths (Anderson *et al.*, 1973; Mclaren *et al.*, 1975; Kozek and Marroquin, 1977; Franz and Büttner, 1983; Cable and Tinsley, 1991; Poddubnaya and Izvekova, 2005; see also Bakke *et al.*, 2006; Morley, 2016 for reviews). Characterising microbiota associated with specific niches of the helminth could help to clarify where these micro-organisms originate from, and the function that they have within the helminth, to build on work achieved by microscopy and imaging. For example, it has been suggested that bacteria in the gut lumen of mongeneans are acquired by ingestion (Cable and Tinsley, 1991) and microbes in the reproductive organs of female nematodes are likely transovarially transmitted endosymbionts (Kozek and Marroquin, 1977). However, such microscopy work is unable to identify the taxonomy of bacteria, and further detail of the microbial genera associated with specific tissues/organs of the helminth, e.g., by laser microdissection (for example, see Ranjit *et al.*, 2006; De Hertogh *et al.*, 2012) of the helminth, would greatly advance the current knowledge.

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

in the current chapter with *S. frederici*, 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 and were present in more than 50% of reads, whereas in the current study Lactobacillaceae constituted only 21.8% of the *H. polygyrus* 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 influenced results.

To summarise, parasitic helminths are associated with a microbiota, which shows intraspecific variation associated with inhabitation of different gut sections. 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 that of the host gut. However, in some instances helminth microbiota diversity exceeds that of the host 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). 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.

5.6 Author Acknowledgements

The manuscript resulting from this chapter is authored by:

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E.L.P: Conceived and designed study, collected, analysed and interpreted data, wrote manuscript.

F.A: Collected data.

M.J.B: Provided guidance regarding processing of microbiota samples.

H.C.H: Involved in conceiving the study and organising field work and logistics, provided comments on each version of the manuscript.

J.R.M: Provided comments on the manuscript.

S.E.P: Conceived and designed study, involved in data interpretation, provided comments on each version of the manuscript.

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 can 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* that were isolated from the faeces of naturally infected wild mice, *Apodemus flavicollis*. On average, significantly more *H. polygyrus* eggs (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. In contrast, there was no significant difference in the probability of *T. muris* egg development between non-self and self faeces, however *T. muris* egg deterioration was signficantly higher in non-self faeces (p = 0.05). These results suggest that faeces provide an extended immune phenotype to the host, and can reduce the development of *H. polygyrus* eggs which the host has already had contact with.

6.2 Introduction

Gut microbiota is vital for immune system development and function; the human foetus, which develops in an almost sterile environment, and germ-free mice, both exhibit immature immune systems, which are able to fully develop following colonisation of the gut by bacteria (Round and Mazmanian, 2009; Weng and Walker, 2013). Microbiota stimulate 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 identified as a stronger driver of specific immune defences than genotype of the host, and the microbiota-associated immune

phenotype can be transferred between individuals by microbiota transplant (Koch and Schmid-Hempel, 2012), and vertical 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 maintain 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 (Bautista-Garfias *et al.*, 2001; Martínez-Gómez *et al.*, 2009; Coêlho *et al.*, 2013). Administration of probiotics which increase the abundance of *Lactobacillus* bacteria in the gut can have 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 also 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 helminth 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). Meanwhile, 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 of 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 resistance to helminths.

In addition to the direct impacts of bacteria on helminth development, host immune responses to parasites may be affected by microbiota (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 of some helminth species such as *Ostertagia circumcincta*, and

inhibiting development (Jørgensen *et al.*, 1998), a response that could be modulated by microbiota (Reynolds *et al.*, 2015). However, faecal composition does not prevent development of all helminths species; the hatchability of nematode eggs from a rabbit host (*Trichostrongylus retortaeformis* and *Graphidium strigosum*) 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).

Here the ability of faeces to act as an extended immune phenotype of the host by inhibiting helminth development is tested. 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 from another randomly selected individual, with a presumably different microbiota composition ('non-self'), and the probability and rate of egg development between culture in self and non-self faeces were compared.

6.3 Materials and methods

6.3.1 Study area and rodent sampling

Live-trapping of *Apodemus 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; see Appendix A.7; Table A.7.1). 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 and the liquid filtrate containing these bacteria was collected and pooled with the previously prepared faecal bacteria solution. The faecal bacteria solution was 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. Eggs isolated on strainers 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 NGM agar, 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, from which eggs were separated using a pipette 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 Table 7.1; 7.2). 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 Table 6.1 and 6.2.

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 5,500 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 Tables 6.1 and 6.2, and Appendix A.7; Table A.7.1 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 Table 6.1), while for *T. muris* five self and nine non-self donor-recipient combinations were made (from which 12 self and 13 non-self cultures were made, including replicates, see Table 6.2). To avoid dehydration, 2 ml of ultra-pure water was added to the bottom of each culture dish, which were then sealed with Parafilm® 'M' and maintained at a constant 23°C in the dark.

Table 6.1: Recipient-donor identity combinations of *Apodemus flavicollis* individuals used in a transplant experiment of *Heligmosomoides polygyrus* eggs. The number of culture replicates for each recipient-donor combination across the course of the experiment is presented.

Recipient identity	Donor identity	Culture type	No. of replicates
Mouse 1	Mouse 1	Self	2
Mouse 2	Mouse 2		1
Mouse 4	Mouse 4		2
Mouse 5	Mouse 5		1
Mouse 6	Mouse 6		3
Mouse 8	Mouse 8		4
Mouse 9	Mouse 9		3
Mouse 2	Mouse 9	Non-self	5
Mouse 3	Mouse 2		1
	Mouse 4		1
	Mouse 6		2
Mouse 5	Mouse 8		2
Mouse 6	Mouse 1		1
	Mouse 8		2
Mouse 7	Mouse 6		1
Mouse 10	Mouse 4		1

Table 6.2: Recipient-donor identity combinations of *Apodemus flavicollis* individuals used in a transplant experiment of *Trichuris muris* eggs. The number of culture replicates for each recipient-donor combination across the course of the experiment is presented.

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

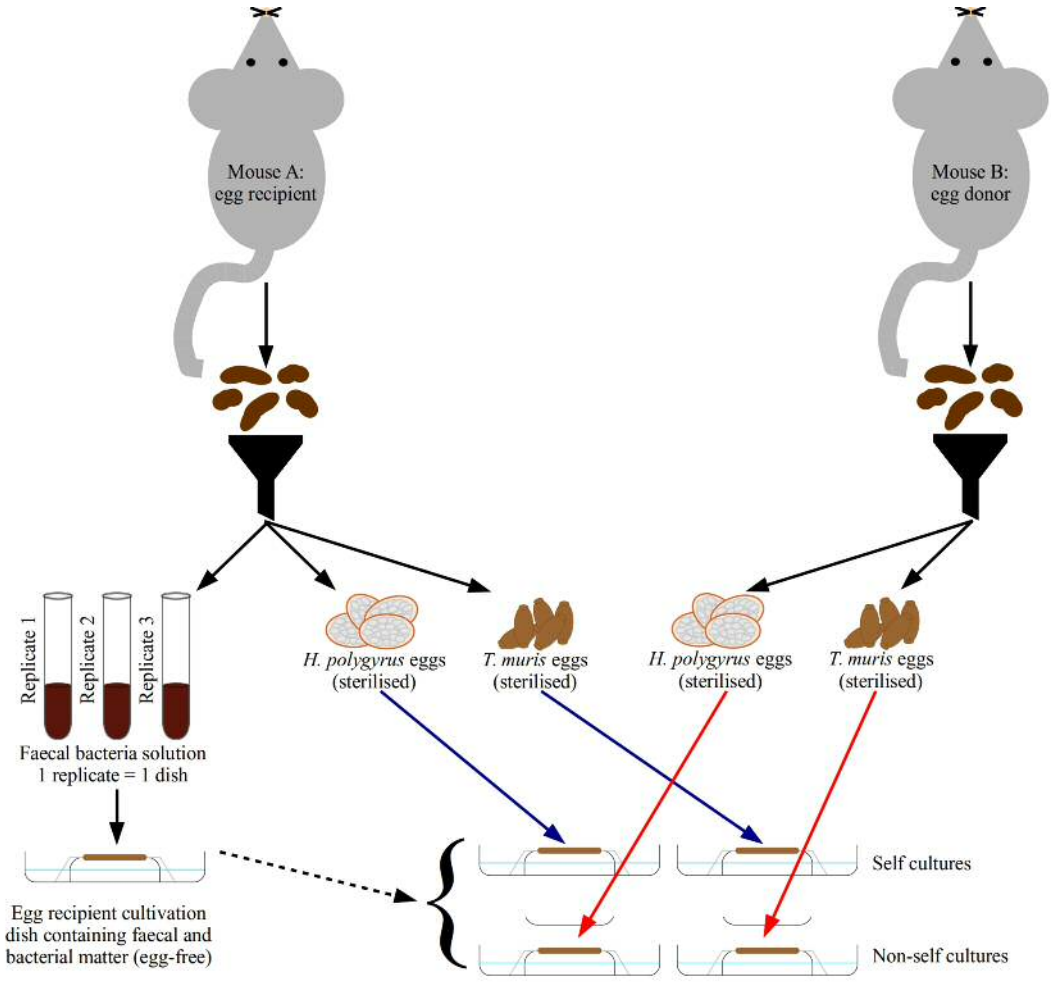

Figure 6.1: Visual representation of experimental design. Each individual or 'egg recipient' was randomly assigned an 'egg donor'. Helminth eggs of *Heligmosomoides polygyrus* and *Trichuris muris* were separated from the faeces of both the egg donor and recipient. Eggs of one helminth species from the 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 1,845 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 (see Fahmy, 1954), 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 host individuals (see Appendix A.7; Table A.7.1), frozen faeces collected 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 (Maurice *et al.*, 2015). Preparation of samples (DNA extraction, DNA quantification, PCR and PCR product purification), and subsequent sequencing of the resulting amplicon library followed methods presented in Chapter 3 (see '*3.3.4 16S rRNA gene sequencing*'). Samples with a final read count 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 (Anders and Huber, 2010; Love *et al.*, 2014).

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 indices that account 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 (mean EPG ± standard error = 29.2 ± 7.5) compared to other species. *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 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. 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).

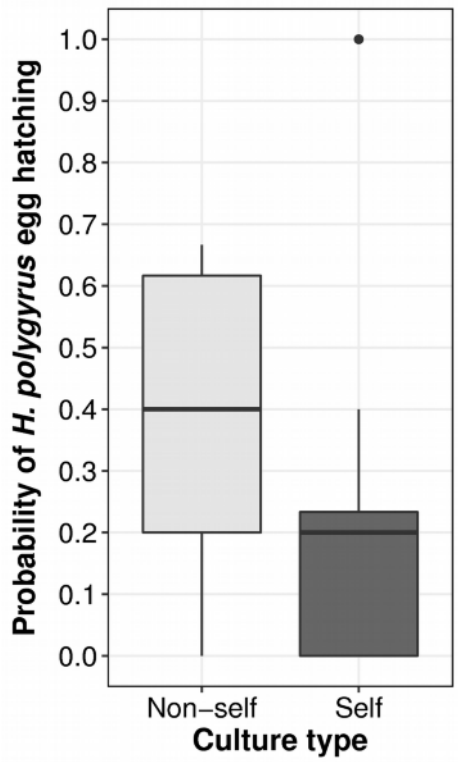

Figure 6.2: Probability of egg hatching of *Heligmosomoides polygyrus* eggs in a transplant experiment, whereby eggs were cultured in 'self' faeces of the host and 'non-self' faeces of a randomly selected individual. Boxes demonstrate the upper and lower quartiles, with median hatching probability indicated.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

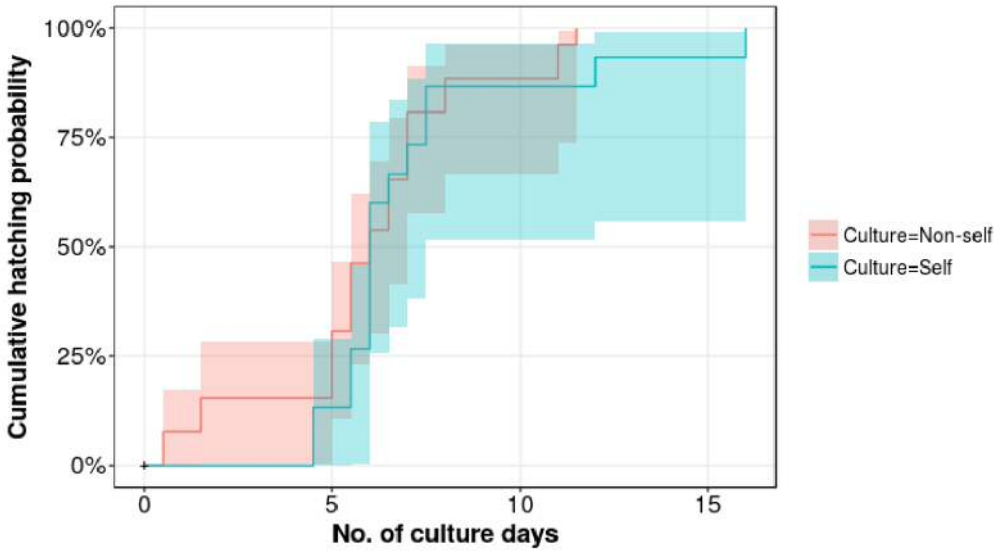

Figure 6.3: Survival plot of cumulative hatching probability of *Heligmosomoides polygyrus* eggs in a transplant experiment, whereby eggs were cultured in 'self' faeces of the host and 'non-self' faeces of a randomly selected individual. Lines represent the expected hatching probability on each day and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

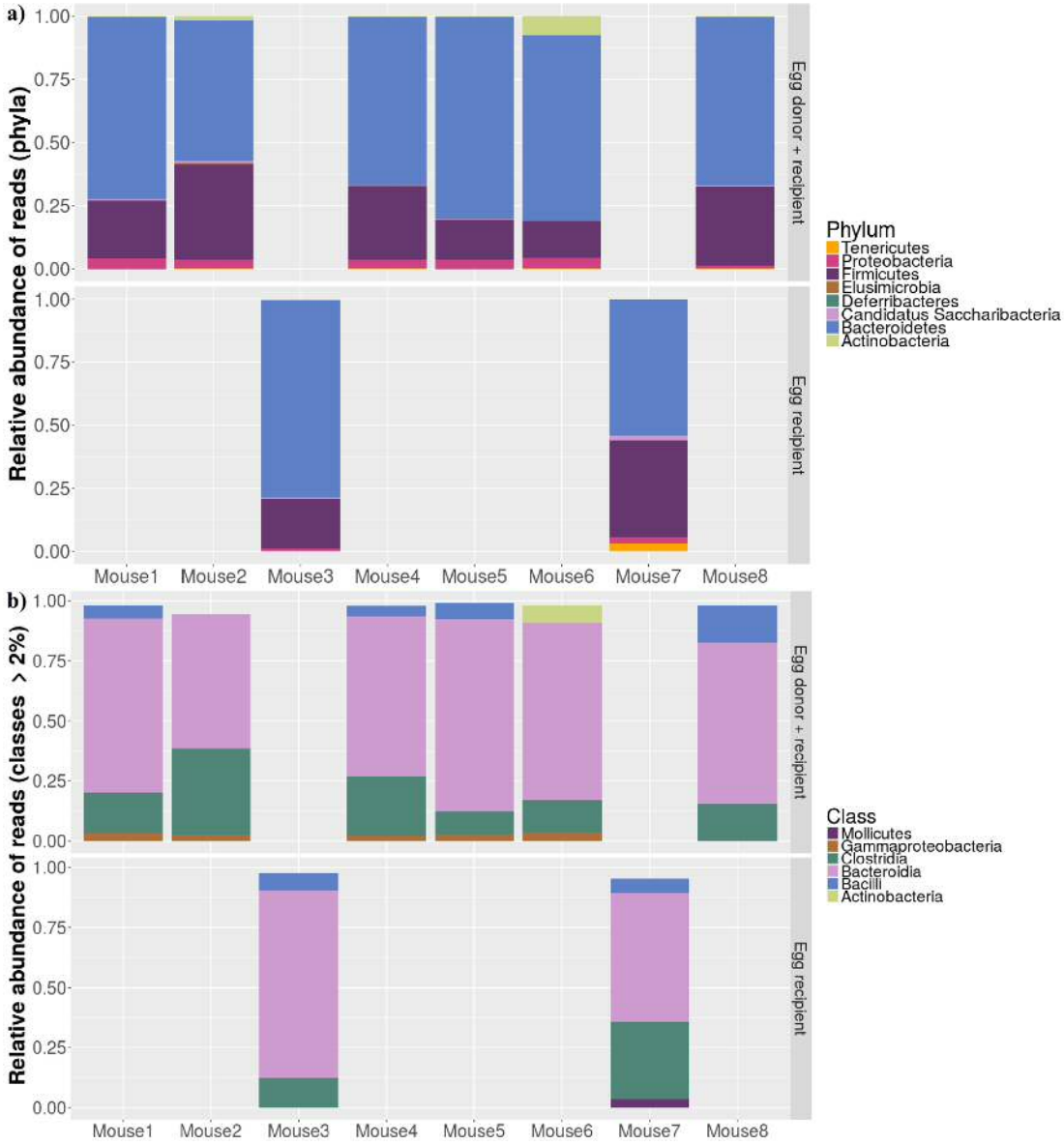

Figure 6.4: Mean proportion of reads of bacterial a) phyla and b) classes (>2%) in faeces of *Apodemus flavicollis* individuals used either as a donor and recipient or only as a recipient of *Heligmosomoides polygyrus* eggs in an egg transplant experiment.

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).

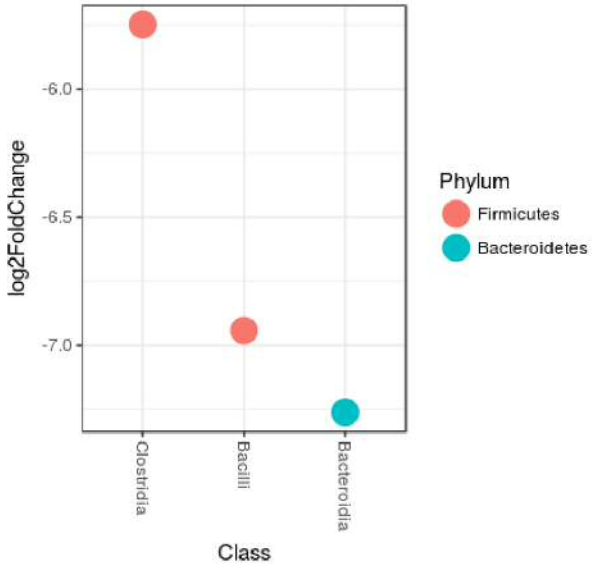

Figure 6.5: OTUs in faecal microbiota that were significantly different in abundance between donor and recipient individuals versus only recipient individuals in an egg transplant experiment with *Heligmosomoides polygyrus* eggs, grouped by microbial class. Briefly, DESeq was used to identify significantly 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.

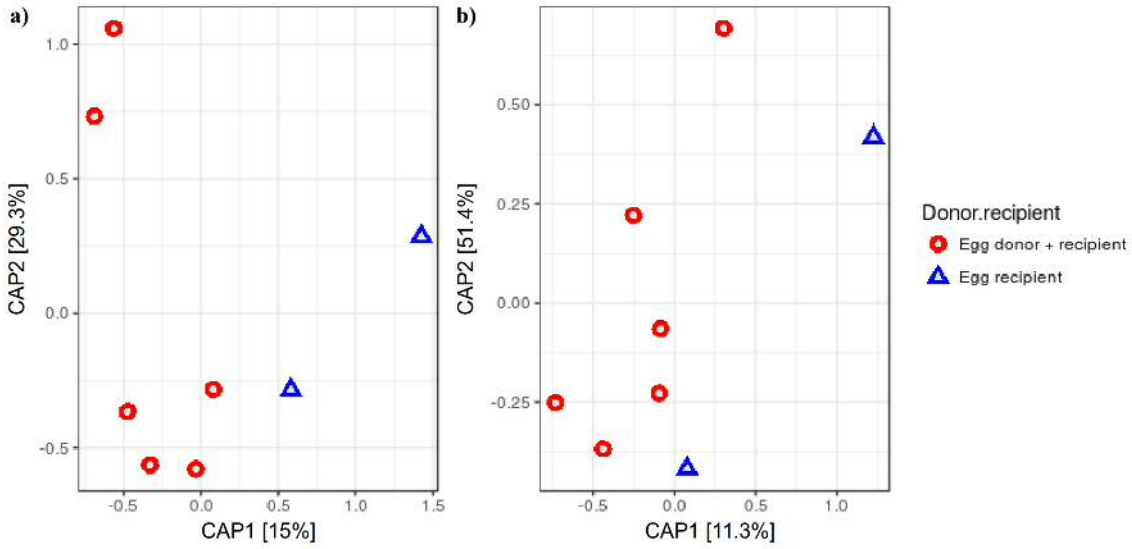

Figure 6.6: Non-metric multidimensional scaling plot of microbiota divergence between faecal samples of individuals used as egg donor and recipients, or only as egg recipient individuals in an egg transplant experiment with *Heligmosomoides polygyrus* eggs based on a) Bray–Curtis (explaining 44.3% variation)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

6.5 Discussion

Mean hatch success was significantly higher in non-self (40.3%) compared to self (20.4%) faeces (Figure 6.2), suggesting that faeces can inhibit *H. polygyrus* egg hatching, but ony those eggs to which the host has had previous contact with. Unsurprisingly, probability of egg hatching was positively associated with time since start of culture. However, other potential influential factors, such as the alpha diversity of faecal microbiota of the egg donor or the recipient microbiota composition, helminth egg burden in faeces from the donor or the recipient, and sex of the donor and recipient, all had no significant effect on hatch success, suggesting that natural variation between individuals was not responsible for differences in egg hatching. There was no significant difference in *T. muris* egg embryonation between self and non-self faeces. However, there was a significantly greater chance of egg deterioration in non-self faeces; 31.5% eggs in these cultures showed visible signs of deterioration compared to 0% in self faeces.

Intraspecific gut microbiota composition varies significantly between individuals due to a myriad of host and environmental characteristics (e.g., Lozupone *et al.*, 2012), including 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 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 individual hosts (Figure 6.6), suggesting that overall microbiota composition was not responsible for differences in helminth development.

Egg shedding and faecal yield varied between individuals, as well as between days for any given individual. In addition, due to the unpredictable and uncontrollable nature of wild animal trapping, sample sizes were small and 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). Consequently, it was not possible to directly compare the difference in egg development between self and non-self faeces for all individuals, and results obtained from small sample sizes should be interpreted with caution. However, the results in the current chapter do suggest that cultivation of surface-sterilised eggs in

non-self faeces (40.3% hatch success) increases the probability of egg hatching compared to other culture techniques; previous studies using trypan blue staining have predicted that *H. polygyrus* egg viability is at least 92%, however mean egg hatching in culture on nematode growth medium is 25.3% (Donskow-Łysoniewska *et al.*, 2013).

In general, potential helminth hosts have evolved a number of specific and non-specific immune responses that may be mediated by the microbiota to prevent helminth 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* (see Pearce *et al.*, 2004), the sheep liver fluke *Fasciola hepatica* (see Moxon *et al.*, 2010), and nematodes such as *Strongyloides venezuelensis* (see Gonçalves *et al.*, 2012), *Ostertagia circumcincta* (see Jørgensen *et al.*, 1998), and other rabbit parasites (see Lambert *et al.*, 2015). Antibodies produced by the host, may be shed in faeces, and can subsequently bind to parasite eggs and affect development of some (e.g., *O. circumcincta*, see Jørgensen *et al.*, 1998), but not all, helminth species (Lambert *et al.*, 2015), which is perhaps why no significant effects on probability of egg development were observed for *T. muris*. Should host antibodies, either present in the faeces or bound to the egg surface, affect *H. polygyrus* egg hatching, external washing and sterilisation of the egg, and introduction into non-self faeces may release eggs from antibodies and the inhibitory action that they induce on egg development, increasing the probability 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 (coprophagy has been observed in laboratory mice,

although there is no evidence for this behaviour in wild *Apodemus flavicollis*; see Ghazal and Avery, 1976).

Although bacteria are 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, helminth 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 in 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 (see 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, it would be disadvantageous for wild *T. muris* to evolve such specific bacterial requirements for hatching as observed in laboratory strains. 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).

Due to the small sample sizes of the current study, results should be interpreted with caution. Similarly, because of variable re-capture rates of individuals, as well as daily variation in egg and faecal shedding, some individuals were sampled as egg donors or recipients more frequently than other individuals, which may have skewed results. Likewise, due to insufficient faeces, faecal egg counts and faecal microbiota analyses were not performed for all individuals. Further investigation could be made into the effect of host faecal microbiota on the development of helminth eggs by transplanting eggs into faeces between individuals harbouring low and high burden infections. Comparing helminth development in faeces from hosts with different burdens may shed light on the common skew of parasite populations which results in 20% of the host population harbouring 80% of parasites (Perkins *et al.*, 2003); highly parasitised individuals may be more susceptible to infection due to differences in faecal microbiota compared to more resistant hosts.

In conclusion, the current study suggests that host faeces may affect 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 to inhibit parasite development.

6.6 Author Acknowledgements

The manuscript resulting from this chapter is authored by:

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K.E.W: Involved in designing study, collected data.

H.C.H: Involved in conceiving the study and organising field work and logistics, provided comments on each version of the manuscript.

J.R.M: Provided comments on the manuscript.

S.E.P: Conceived and designed study, involved in data interpretation, provided comments on each version of the manuscript.

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) also 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 coinfected 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 or conceptualise how the microbiota and macrobiota interact (e.g., Hayes *et al.*, 2010; Walk *et al.*, 2010; Bancroft *et al.*, 2012; Cooper *et al.*, 2013; Glendinning *et al.*, 2014; Kreisinger *et al.*, 2015; Reynolds *et al.*, 2015 Hayes *et al.*, 2010; Walk *et al.*, 2010; Cooper *et al.*, 2013; Kreisinger *et al.*, 2015; please see Chapter 1 for more comprehensive list of references), the number of studies on this topic are currently relatively few, despite a plethora of research indicating that, individually, these two communities each have 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. 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 which 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 implications on the whole gut biome. Due to recent advances in technologies enabling research on microbial communities (Marchesi and Ravel, 2015), researching microbiota-macrobiota interactions using next generation methods is now feasible for many laboratories. This thesis uses an ecological approach to tease apart some of these microbiota-macrobiota interactions in wild

rodents, 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 to a carnivorous diet (Zhu *et al.*, 2011). In addition, wild animals can provide a model system, which unlike laboratory animals, harbour a diverse microbiota in terms of both the OTUs present and microbiota variation between individuals. Furthermore, wild animals are exposed to a range of intrinsic and extrinsic factors, rendering study 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 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 almost 13% 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 literature gaps by studying both the microbiota and macrobiota components of the gut biome, and how they interact. However, to study other components of the gut microbiota is currently more challenging; for example, there are no universal primers for viruses as there are for bacteria, thus comprehensive characterisation of the virome is time consuming and costly (Wylie *et al.*, 2015). However, like 16S rRNA bacteria sequencing, technologies for virome characterisation are improving (Wylie *et al.*, 2015).

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, which included 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. Interestingly, more variation in microbiota composition and OTU abundances between pre- and post-treatment individuals were observed in the control group than in the anthelmintic group. As samples were collected over the course of five months, it is possible that the microbiota changes between pre- and post-treatment in the control group were natural fluxes driven by seasonality, e.g., changing food availability (Maurice *et al.*, 2015). In addition, it could be speculated that anthelmintic treatment inhibited seasonal variation in the microbiota, although other factors, such as animal stress through handling, stochastic differences between individuals and treatment groups cannot be discounted as other potential drivers of microbiota differences in the anthelmintic and control groups.

Another study which claimed to completely clear an experimental helminth infection using anthelmintic (however no data were provided in the published article to confirm 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 to microbiota that are associated with helminth infection (which could be lost if helminths are completely eradicated, as suggested by Houlden *et al.*, 2015), helminth treatment approaches could avoid current mass drug administration to 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*; see Valanparambil *et al.*, 2014, and *T. muris*; see 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 faeces from untreated individuals. Results may establish if reduction in helminth burden associated with

anthelmintic treatment is also associated with changes in the development of progeny, which may also impact transmission events. By understanding if the composition of faecal microbiota following anthelmintic infection affects helminth development, strategies could be employed during treatment regimes to avoid further parasite transmission, such as faecal clearing of pastures with recently treated livestock (Corbett *et al.*, 2014).

Investigating how dietary anthelmintics affect microbiota could help us to further understand how helminth removal and anthelmintics affect the gut microbial community, particularly in wild animals. 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 has 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 responses (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). Diet, including consumption of 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). However, it is currently unknown if the anthelmintic effect of tannins, or the tannins themselves, are linked to changes in microbiota following consumption. 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 routinely administered to treat bacteria 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 a positive effect on prevalence and fecundity of helminths. Chapter 4 exemplifies the need for long-term and detailed studies on the effect of antibiotic (and anthelmintic) treatments on components of the gut biome other than those being intentionally targeted by the treatment: although in previous studies antibiotics initially appeared to be an effective method of treating 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, the eggs shed in the environment by helminths within anthelmintic-treated hosts 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, the subsequent infective larvae inoculated into hosts, and establishment of infection confirmed.

Future work to complement Chapter 4 could include testing the effect of individual antibiotics on the helminth community. It was appropriate in Chapter 4 to initially test the effect of a five antibiotic cocktail on the helminth community; wild, treated mice were subjected to a natural, bacteria-rich environment, that would rapidly repopulate the microbiota between the weekly administered doses of antibiotic (compared to humans or livestock, who are usually administered a daily course of antibiotics). The data provide good evidence that the topic of antibiotic and helminth interactions are worthy of study. However, there are very few real-life situations in which five different antibiotics would be administered simultaneously, and investigating the effect of single antibiotics on a helminth community would not only provide data more applicable to humans and livestock, but may also help to determine more specifically what drives these changes; whether it be specific ingredients within different antibiotics, or removal of certain bacterial groups. In addition, while ampicillin, vancomycin and neomycin (which comprised three of five of the antibiotic cocktail) are considered important antibiotics to human and animal medicine, and cover three main classes of antibiotic, there are also many other antibiotics that are

commonly administered to animals and humans, and which may pose an environmental risk, that should also be considered for study (for example, see Kemper, 2008), however sample sizes and experimental design should be carefully considered to avoid the risk of antibiotic resistance in the environment (Kemper, 2008).

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. It is somewhat surprising that, to date, only two other studies have used a culture-independent method to characterise the microbiota associated with parasitic helminths, given that there is a growing body of literature on the microbiota of other parasitic species (mainly biting ectoparasites), such as ticks (Carpi *et al.*, 2011) and mosquitoes (e.g., Dong *et al.*, 2009; Chandel *et al.*, 2013). It has long been acknowledged that parasitic helminths are associated with bacteria, including intracellular symbionts (Anderson et al. 1973; Mclaren *et al.*, 1975; Kozek and Marroquin, 1977; Franz and Büttner, 1983; Cable and Tinsley 1991 see also Bakke et al. 2006; Morley 2016 for reviews), while filarial nematodes harbour

Wolbachia spp. infections (Taylor *et al.*, 2005; Duron and Gavotte, 2007; Foster *et al.*, 2014). In other parasites, mosquito species in particular, gut microbiota has been exploited as possible means of biocontrol (Dong *et al.*, 2009; Boissière *et al.*, 2012), and while studies on filarial nematodes have investigated how antibiotics that target *Wolbachia* can eliminate infection from within treated hosts (Bandi *et al.*, 2001; Taylor *et al.*, 2005), this is still an underexploited area of research. The characterisation of microbiota (as achieved in Chapter 5) from more helminth species, particularly those of veterinary or medical significance, could inform potential experiments by which to treat helminth infection using antibiotic (following Chapter 4), as a future revenue of helminth control.

In Chapter 5 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, but instead undergoes community assembly (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, 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.

Further work could also investigate microbiota associated with specific niches of the helminth. In Chapter 5, helminths that were sampled for microbiota analyses first underwent a series of washing steps in a buffer solution (TBS), which is likely to have removed some excess bacteria from the host gut (as demonstrated by the fact that microbiota composition significantly differed between host gut section and that of the helminths therein). However external bacteria no doubt would of remained on the helminth. It could be argued that, much like the mammalian skin microbiota (e.g., Cogen *et al.*, 2008; Belkaid and Segre, 2014), or mucosal microbiota of amphibians (Colombo *et al.*, 2015) and fish (Lazado and Caipang, 2014), the microbiota associated with the external surface of the helminth is of importance to helminth survival and internal functions. However, in future studies, prior to bacterial sequencing, helminths could be surface sterilised (perhaps using techniques adapted from Chapter 6 for egg sterilisation), in order to characterise just those bacteria associated with the internal structures of the helminth. Likewise, although microbiota characterisation of specific helminth tissue would not be possible for the likes of *Hymenolepis* spp., which are mainly composed of a nutrient-absorbing tegument, and thus lack a digestive system (Lumsden, 1975), laser microdissection (for example, see Ranjit et al. 2006; De Hertogh et al. 2012) could be employed to isolate microbiota from specific tissues of other helminth species. Understanding where bacteria are located within helminths may shed light on how/where bacteria associated with helminths is acquired (e.g., Cable and Tinsley, 1991), as well as their function within the helminth.

Much of the previous work on microbiota-macrobiota interactions has 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 by affecting helminth development, by transplanting eggs into faeces from non-self and self individuals, and monitoring the subsequent 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 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).

It is important to emphasise that sample sizes in Chapter 3, 4, 6 and to some extent also 5, are very low, as a result of small rodent populations within the study sites. In addition, despite efforts to provide repeat data by sampling mice from two different locations (Cavedine and Pietramurata), the population size at Pietramurata was low. Small sample sizes not only risks that statistical models (including GLMMs) are overfitted (Subramanian and Simon, 2013), but are also especially problematic when analysing parasite data, as parasite infections are typically distributed

throughout the host population such that 20% of individuals harbour 80% of the parasite burden (Perkins *et al.*, 2003). Consequently, data obtained from a small population size can be dramatically skewed by just a few heavily parasitised individuals. In addition, the destructive nature of gut microbiota and adult helminth sampling meant that it was only possible to sample these parameters at a single time point (at either pre- or post-treatment) for a given individual, with the consequence that stochastic variation between individuals may have affected results. Both the helminth and microbial community of the European shag (*Phalacrocorax aristotelis*) gut have been successfully characterised using non-destructive endoscope technologies (Newbold *et al.*, 2017), allowing microbiota and macrobiota to be described for a given individual over multiple time points, and is a method that could be further exploited for future microbiota-macrobiota studies (for some animal species) to overcome the limitations associated with destructive sampling. Data presented within this thesis could also be advanced by combining the current qualitative data on microbiota composition with that of quantitative data, for example using qPCR techniques, to quantify how the absolute abundances of OTUs change following treatment.

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). 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 faecal 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	N/A	N/A	N/A	N/A	Bacteria &	(Berg Miller *et al.*, 2012)

			composition					viruses	
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-infectious disease	Immunity	N/A	Bacteria	(Bull-Otterson *et al.*, 2013)
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	N/A	N/A	N/A	Bacteria	(Devine *et al.*, 2013)

				procedure					
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	N/A	N/A	N/A	N/A	Bacteria &	(Dudek *et al.*, 2014)

			composition					archaea	
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 &	(Fernando *et al.*, 2010)

								archaea	
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 & funghi	(Geurden *et al.*, 2014)
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	Domestication	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	Domesticatio n	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	N/A	N/A	N/A	Bacteria	(Pissavin *et al.*, 2012)

				composition					
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 & archaea	(Queipo-Ortuño *et al.*, 2013)

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	Environment	N/A	N/A	N/A	Bacteria	(Roeselers *et al.*, 2011)

			composition						
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	Phylogeny	Interspecific	N/A	N/A	N/A	Bacteria	(Sanders *et al.*, 2014)

		mammal		comparison					
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-	Immunity	Diet	Prebiotic	N/A	Bacteria	(Serino *et al.*, 2011)

			infectious disease						
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,	(Torok *et al.*, 2014)

								funghi & protozoa	
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 comparison	Diet	N/A	N/A	N/A	Bacteria	(Vasaï *et al.*, 2014a)
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	N/A	N/A	Bacteria	(Wang *et al.*, 2016)

				transmission					
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 disease	N/A	N/A	N/A	Bacteria	(Wu *et al.*, 2014b)
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-infectious disease	N/A	N/A	N/A	N/A	Bacteria	(Zhu *et al.*, 2014)
Model	Perturbation	Mammal	Diet	Age	N/A	N/A	N/A	Bacteria	(Zhu *et al.*, 2015)
Domestic	Observation	Mammal	Infectious	N/A	N/A	N/A	N/A	Bacteria	(Zinicola *et al.*, 2015)

	disease

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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	$\log^2$ fold	DESeq	*p*-value	Adjusted	Phylum	Class

		change	change standard error	statistic		*p*-value		
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 change	Log^2 fold change standard error	DESeq statistic	*p*-value	Adjusted *p*-value	Phylum	Class
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**

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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