



HAL
open science

Increasing helminth infection burden depauperates the diversity of the gut microbiota and alters its composition in mice

Emmanuel Guiver, Maxime Galan, Cédric Lippens, Jérôme Bellenger, Bruno Faivre, Gabriele Sorci

► **To cite this version:**

Emmanuel Guiver, Maxime Galan, Cédric Lippens, Jérôme Bellenger, Bruno Faivre, et al.. Increasing helminth infection burden depauperates the diversity of the gut microbiota and alters its composition in mice. *Current Research in Parasitology & Vector-Borne Diseases*, 2022, 2, pp.100082. 10.1016/j.crpvbd.2022.100082 . hal-03620254

HAL Id: hal-03620254

<https://hal.science/hal-03620254>

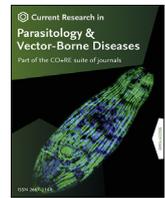
Submitted on 19 May 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Increasing helminth infection burden depauperates the diversity of the gut microbiota and alters its composition in mice

Emmanuel Guiver^a, Maxime Galan^b, Cédric Lippens^a, Jérôme Bellenger^c, Bruno Faivre^{a,2}, Gabriele Sorci^{a,*,2}

^a Biogéosciences, CNRS UMR 6282, Université de Bourgogne Franche-Comté, 6 Boulevard Gabriel, 21000 Dijon, France

^b Centre de Biologie pour la Gestion des Populations, CBGP, INRA, CIRAD, IRD, Montpellier SupAgro, Université de Montpellier, 755 Avenue du Campus Agropolis, CS 30016, 34988 Montpellier-sur-Lez Cedex, France

^c Lipides Nutrition Cancer, INSERM UMR 1231, Université de Bourgogne Franche-Comté, 6 Boulevard Gabriel, 21000 Dijon, France

ARTICLE INFO

Keywords:

Dysbiosis
Genotype × environment interaction
Heligmosomoides polygyrus
Host genotype
Nematode

ABSTRACT

The gut microbiota constitutes a diverse community of organisms with pervasive effects on host homeostasis. The diversity and composition of the gut microbiota depend on both intrinsic (host genetics) and extrinsic (environmental) factors. Here, we investigated the reaction norms of fecal microbiota diversity and composition in three strains of mice infected with increasing doses of the gastrointestinal nematode *Heligmosomoides polygyrus*. We found that α -diversity (bacterial taxonomic unit richness) declined along the gradient of infective doses, and β -diversity (dissimilarity between the composition of the microbiota of uninfected and infected mice) increased as the infective dose increased. We did not find evidence for genotype by environment (host strain by infective dose) interactions, except when focusing on the relative abundance of the commonest bacterial families. A simulation approach also showed that significant genotype by environment interactions would have been hardly found even with much larger sample size. These results show that increasing parasite burden progressively depauperates microbiota diversity and contributes to rapidly change its composition, independently from the host genetic background.

1. Introduction

The gut of vertebrates is one of the taxonomically and functionally most diverse ecosystems on earth, with billions of microbes, mainly bacteria, interacting with each other and with host cells (The Human Microbiome Project Consortium, 2012). Given the importance of the gut microbiota for host homeostasis (LeChatelier et al., 2013; Belkaid and Hand, 2014), a particular effort has been devoted to understanding the processes underlying the maintenance of a diverse and healthy microbiota (Lozupone et al., 2012).

Infection with gastrointestinal helminths is among the environmental factors likely to affect the diversity and the composition of the gut microbiota (Glendinning et al., 2014; Gause and Maizels, 2016; Zaiss and Harris, 2016). The effect of infection with gastrointestinal helminths has been investigated in several host species, with human and rodent studies being the most numerous (Walk et al., 2010; Lee et al., 2014; Holm et al., 2015; Houlden et al., 2015; Kreisinger et al., 2015; Cattadori et al., 2016; Jenkins et al., 2017; Wegener Parfrey et al., 2017; Yang et al., 2017;

Jenkins et al., 2018a,b; Martin et al., 2018; Rosa et al., 2018; Schneeberger et al., 2018; Su et al., 2018). This work has provided mixed results since positive, negative or no effects have been reported. There are several possible explanations for this discrepancy. First, it might prove difficult to compare studies that used different helminth species, because they may have different ecological requirements, differently altering the intestinal ecosystem. In the same line, hosts with different genetic backgrounds might provide worms and gut microbes with different “habitat” features that may affect the outcome of helminth-microbiota interactions. Experimental work has shown that, while infection with the nematode *Heligmosomoides polygyrus* induces an increased abundance of *Lactobacillus* bacteria in the duodenum and ileum of C57BL/6 mice (Walk et al., 2010; Reynolds et al., 2014), a decrease occurs if BALB/c mice are infected (Reynolds et al., 2014; Su et al., 2018). This suggests that the effect of helminth infection on the gut microbiota might be host genotype dependent, and therefore that genotype by environment (G × E) interactions might be important determinants of microbiota diversity.

* Corresponding author.

E-mail address: gabriele.sorci@u-bourgogne.fr (G. Sorci).

¹ Shared senior authors.

While the effect of environmental conditions and host genetic background on the diversity and composition of the gut microbiota has been investigated (see [Spor et al., 2011](#) for a review), how the microbiota composition of a given host genotype changes when exposed to a gradient of infection conditions (i.e. host genotype \times infection burden interactions) remains unexplored. To fill this gap, we performed an experiment where three mouse strains were exposed to increasing infective doses of the gastrointestinal nematode *H. polygyrus* (four environmental conditions), a natural parasite of rodents. The three mouse strains were chosen because they have different patterns of resistance to *H. polygyrus*, and different expression of local (intestinal) immune effectors (innate and acquired, humoral and cellular) ([Filbey et al., 2014](#)). Given that the immune response plays a role in defining the intestinal ecosystem where both helminths and the microbiota strive, we deemed that these three host strains were ideal candidates to assess the importance of genotype \times environment interactions for shaping the diversity and composition of the microbiota. We assessed the diversity and composition of the microbiota and investigated whether host strain and infective dose had additive (parallel reaction norms) or synergistic ($G \times E$) effects on α - and β -diversity of the microbiota, as well as on the relative abundance of the commonest bacterial families. If increasing infection burden with *H. polygyrus* induces a perturbation of the intestinal ecosystem, we predict that high burden should correlate with a loss of diversity and a change in the composition of the microbiota. If the perturbation of the intestinal ecosystem is due to the mounting of an anti-*H. polygyrus* immune response ([Su et al., 2018](#)), we also predict that the loss of diversity and the compositional changes of the microbiota should be host-strain dependent, resulting in $G \times E$ interactions.

2. Materials and methods

2.1. *Heligmosomoides polygyrus* infection

BALB/c ($n = 20$), CBA ($n = 20$) and SJL ($n = 20$) female mice were purchased from JanvierLabs (JRJ, Le Genest-Saint-Isle, France), and housed in plastic cages (18.5 \times 38 \times 22.5 cm) enriched with shelters at the Université de Bourgogne, France. Mice were maintained under a constant temperature (24 °C) and a photoperiod of 12:12 h (light:dark). They were fed *ad libitum* with standard mouse pellets and had *ad libitum* access to filtered tap water. At the age of 7 weeks, mice were infected with L3 larvae of the intestinal nematode *H. polygyrus bakeri*. Five mice per strain received, by gavage, 200, 400 or 600 larvae in 0.1 ml of drinking water. Control, non-infected individuals, received the same volume of water with no larvae. Therefore, the experiment included 12 groups (3 host strains \times 4 infective doses). Six mice died (or were humanely euthanized because they reached the end point, 2 CBA and 4 SJL), which reduced the sample size to 54 individuals. Further details on the experimental design and the effect on host and parasite life history traits can be found in [Lippens et al. \(2016\)](#).

2.2. Microbiota analysis

We collected fecal pellets at day 7, 11, 14 and 28 post-infection. To this purpose, each mouse was set in a clean cage for 10 min. Feces were collected using sterile tweezers, placed in microtubes, immediately frozen and subsequently stored at -80 °C for further molecular analyses. Nevertheless, not all mice produced feces during the 10-min period, at each sampling time (24 mice were sampled 4 times, 16 mice 3 times, 9 mice twice, and 5 once). Sterile microtubes, opened during the feces sampling, were used as negative controls, to assess any potential environmental contamination.

We extracted bacterial DNA from fecal samples using Qiamp stool kit (Qiagen, Hilden, Germany) following the protocol of the manufacturer. We amplified a 251-bp fragment of the V4 region of the 16S rRNA bacterial gene using a slightly modified version of the dual-index method

([Kozich et al., 2013](#); [Galan et al., 2016](#)). Detailed information on the methods used to assess the diversity and composition of the gut microbiota is provided in Supplementary file 1 (see also [Faivre et al., 2019](#)).

2.3. Statistical analyses

We used linear mixed effects models to explore how microbiota diversity and composition was affected by mouse strain and *H. polygyrus* infective dose. We investigated α -diversity, β -diversity, and relative abundance of bacterial families. For each of these response variables, the model included mouse strain (a class variable), infective dose (a continuous variable), and the two-way interaction as fixed effects, and mouse identity as a random effect.

α -diversity was assessed using two metrics: operational taxonomic unit (OTU) richness and Shannon index. To model β -diversity, we computed (i) the mean dissimilarity (Bray-Curtis and unweighted Uni-Frac distances) between the microbiota of infected individuals at each infective dose and the microbiota of non-infected mice; and (ii) the mean dissimilarity between the microbiota of non-infected mice. Models on relative abundance of bacterial families were restricted to the commonest taxa (5 families with relative abundance $> 10\%$), to avoid the risk of spurious results when using rare taxa, occurring in a small number of individuals. However, despite having a low relative abundance (1.67%), we also included *Lactobacillaceae* in our analysis, because we had *a priori* reasons to think that *Lactobacilli* may play a role during *H. polygyrus* infection ([Walk et al., 2010](#); [Reynolds et al., 2014](#); [Su et al., 2018](#)).

All linear mixed effect models were run using PROC MIXED in SAS and degrees of freedom were approximated using the Satterthwaite method. To infer the amount of variance accounted for the fixed effects included in the models we used two approaches. First, we used the method proposed by ([Nakagawa and Schielzeth, 2013](#)), which gives an estimate of the total amount of variation explained by fixed effects. Secondly, in order to assess the relative contribution of each fixed effect, we ran ANCOVA models where α -, β -diversity and relative abundances were averaged for each individual mouse. Therefore, in these models, each mouse had a single observation, allowing us to use ANCOVA models. We used PROC GLM with the “*effects*” option to estimate the proportion of variation (partial η^2) explained by host strain, infective dose and the two-way interaction.

Failure to report statistically significant $G \times E$ interactions is often due to limited statistical power, which might prevent to draw strong conclusion on whether genotypes do have different reaction norms. To address this issue, we conducted a retrospective power analysis. The aim of this analysis was two-fold. First, we wished to know how strong the interaction would have been to get statistical support with the available sample size (54 mice, 167 total observations). To this purpose, we simulated 100 datasets with the same number of mice and observations of the actual dataset. For each α - and β -diversity metric, the simulated data were randomly drawn from a normal distribution with mean and standard deviation corresponding to the actual values. For each of these 100 datasets, we ran a mixed effects model as described above, and we checked whether the host strain \times infective dose interaction was statistically significant. We deemed that if none of these simulated datasets returned a P -value < 0.05 , this might be seen as an indication that the actual sample size was too low to detect a significant host strain \times infective dose interaction. The second aim was to identify the sample size that would have been needed to have 80% likelihood to get a statistically significant $G \times E$ interaction, provided the strength of the interaction reported by the model on the actual data does not change with the sample size. To this purpose, for each α - and β -diversity metric, we randomly drawn 100 values from a uniform distribution bounded between ± 1 standard deviation (computed on the actual data). These values were subsequently subtracted from the actual data, to obtain the simulated data. Therefore, these simulated data represent slight deviations from the actual data, ensuring that the magnitude of the interaction effect did not change when increasing the sample size. The sample

size (number of individual mice and total number of observations) was increased up to 4 times the actual sample size (i.e. 216 mice and 668 observations). For each of the simulated dataset, we ran a mixed effects model as described above and computed the percentage of models with *P*-values below 0.05 for the infective dose × host strain interaction. We considered that if with 216 mice and 668 observations the likelihood to get a significant *G* × *E* interaction was still low (< 80%), we could reasonably conclude that evidence in support to *G* × *E* interactions was at best weak.

3. Results

3.1. α-diversity

The number of OTUs declined with increasing infection burden, as shown by the negative sign of the infective dose estimate (Table 1; Fig. 1A). The number of OTUs also varied among host strains, with BALB/c mice harboring a richer microbiota compared to the two other strains (Fig. 1A; Table 1; Supplementary Table S1). The interaction between host strain and infective dose was not statistically significant, suggesting similar reaction norms of number of OTUs for the different host strains (Table 1). An ANCOVA model run on average values per mouse, indicated similar proportion of variance explained by host strain (29.4%) and infective dose (24.7%), while only 4% of total variance was accounted by the interaction.

When using the Shannon index, we found that BALB/c mice harbored a more diverse microbiota compared to the two other strains, while neither the infective dose neither the host strain × infective dose interaction were statistically significant (Table 1; Supplementary Fig. S1A; Supplementary Table S1). Host strain explained the largest fraction of variance (29.2%), while infective dose and the interaction accounted for only 2.2% and 9.4% of total variance, respectively.

Lack of support for the host strain × infective dose interaction may stem from a small sample size and the associated low statistical power. To investigate this point, we ran linear mixed effects models on simulated datasets (see Section 2.3. for details). In four out of 100 models run on simulated OTU richness (and in six models on simulated Shannon index), we found that the *G* × *E* interaction was statistically significant (at the 0.05 significance threshold), showing that our sample size was large enough to detect significant *G* × *E* interactions, provided the differences in the reaction norms were large enough (e.g. crossing reaction norms; see Supplementary Fig. S2A). Assuming that the estimate of the *G* × *E* interaction obtained with the available sample size does not change when increasing the number of mice sampled, the simulations showed that even with a four time larger sample size, the probability to find a statistically significant *G* × *E* interaction was well below the 80% threshold for the number of OTUs (57% of models with *P* < 0.05, Supplementary Fig. S3A). When analyzing the Shannon index, a sample size three times larger than the current one would have been necessary to have a satisfactory likelihood to get a statistical support for the *G* × *E* interaction (89% of models with *P* < 0.05, Supplementary Fig. S3B).

3.2. β-diversity

The dissimilarity of the microbiota between uninfected and infected mice steadily increased as the infective dose increased (Table 2; Fig. 1B; Supplementary Fig. S1B). Microbiota dissimilarity also varied among the three host strains (Fig. 1B, Supplementary Fig. S1B; Supplementary Table S1). However, the rate of increase of microbiota dissimilarity along the gradient of infection burden was similar for the three host strains, as indicated by the non-significant infective dose × host strain interaction (Table 2). Whatever the β-diversity metric used, infective dose explained, by far, the largest amount of variance (Bray-Curtis, 72.4%, UniFrac, 70.2%). Host strain (10.1%, 13.7%) and the interaction (5.7%, 9.3%) explained a much smaller fraction of total variance.

Table 1 Linear mixed effects models investigating the effect of host strain and *H. polygyrus* infective dose on α-diversity (OTU richness and Shannon index) of the fecal microbiota. For each metric, we ran two models, the full model and the model with no host strain × infective dose interaction. To estimate the host strain effect, the SJL mouse strain was set as reference. Each model also included mouse ID as a random factor. The marginal *R*² was 39.2% and 35.9% for OTU richness and Shannon index, respectively

OTU richness	Shannon index			
	Type 3 tests of fixed effects		Type 3 tests of fixed effects	
	Estimate (SE)	<i>F</i>	<i>df</i>	<i>P</i>
Model with interaction				
Intercept	161.75 (5.22)	9.94	2, 161	<0.0001
Host strain (BALB/c)	18.36 (6.39)			
Host strain (CBA)	-6.22 (6.90)			
Infective dose	-0.032 (0.014)	15.28	1, 161	0.0001
Host strain × infective dose	0.018 (0.017)	1.19	2, 161	0.3079
Host strain (BALB/c) × infective dose				
Host strain (CBA) × infective dose	-0.004 (0.018)			
Model with no interaction				
Intercept	159.62 (3.71)	45.88	2, 163	<0.0001
Host strain (BALB/c)	23.78 (3.81)			
Host strain (CBA)	-7.50 (4.12)			
Infective dose	-0.025 (0.007)	13.64	1, 163	0.0003
	Estimate (SE)	<i>F</i>	<i>df</i>	<i>P</i>
Intercept	4.740 (0.099)	7.31	2, 60.3	0.0014
Host strain	0.414 (0.122)			
Host strain (BALB/c)	0.086 (0.131)			
Infective dose	-0.0001 (0.0003)	1.31	1, 56	0.2566
Host strain × infective dose	0.0002 (0.0003)	2.26	2, 53.5	0.1146
Host strain (BALB/c) × infective dose				
Host strain (CBA) × infective dose	-0.0004 (0.0004)			
	Estimate (SE)	<i>F</i>	<i>df</i>	<i>P</i>
Intercept	4.744 (0.072)	36.10	2, 58.6	<0.0001
Host strain	0.486 (0.075)			
Host strain (BALB/c)	-0.033 (0.081)			
Infective dose	-0.0001 (0.0001)	0.71	1, 51.7	0.4049

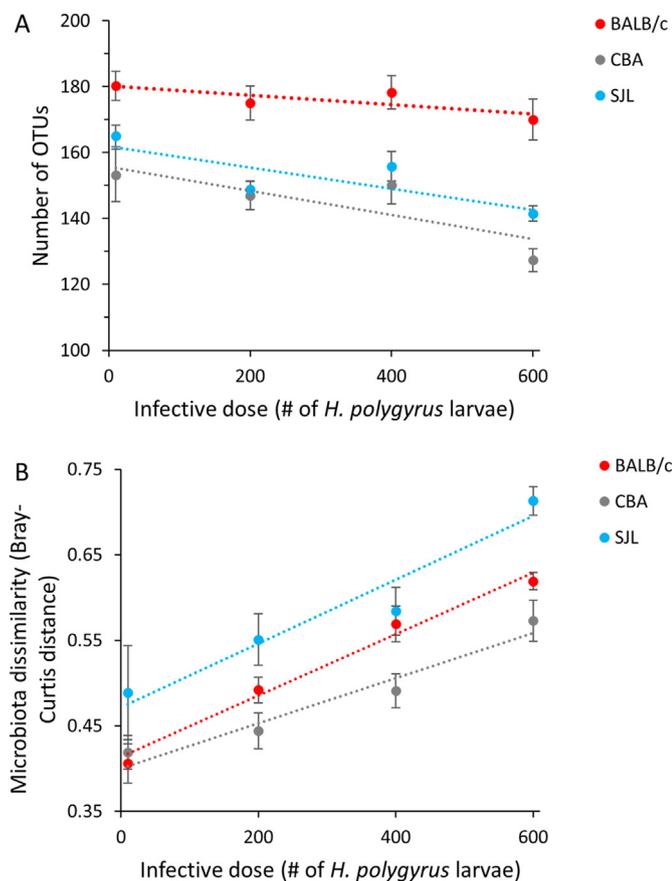


Fig. 1. Reaction norms of OTU richness (A) and dissimilarity (Bray-Curtis distances) (B) of the fecal microbiota in three strains of mice infected with an increasing number of *Heligmosomoides polygyrus* larvae. We report the dissimilarity between the composition of the microbiota of uninfected and infected (200, 400 and 600 *H. polygyrus* larvae) mice. The values for 0 larvae refer to the inter-individual dissimilarity in the microbiota of uninfected mice. We report means \pm standard errors. Dotted lines refer to the fit of the linear mixed effects models.

A retrospective power analysis showed that the available sample size was large enough to detect significant $G \times E$ interactions, provided the strength of the interaction was strong (crossing reaction norms; [Supplementary Fig. S2B](#)), (three out of 100 models on simulated Bray-Curtis dissimilarity had a $G \times E$ interaction with a P -value lower than 0.05, and two on unweighted UniFrac dissimilarity). Assuming that the estimate of the $G \times E$ interactions obtained with the available sample size does not change when increasing the number of mice sampled, even with four times larger sample size, the probability to get a statistically significant $G \times E$ interaction was much lower than 80% when using the Bray-Curtis dissimilarity (56% of models with $P < 0.05$, [Supplementary Fig. S4A](#)). When using the UniFrac dissimilarity, a three times larger sample size would have been required to reach the 80% likelihood to detect a significant $G \times E$ interaction (82% of models with $P < 0.05$, [Supplementary Fig. S4B](#)).

3.3. Bacterial relative abundance

OTUs were assigned to 62, 54 and 47 bacterial families for BALB/c, CBA and SJL strains, respectively. We found a statistical support for a host strain \times infective dose interaction for *Rikenellaceae*, *Lactobacillaceae* and *Bacteroidaceae*, although for this latter taxon, the P -value was rather close to the 0.05 threshold ([Fig. 2](#); [Table 3](#); [Supplementary Table S2](#)), with proportion of variance accounted by the interaction being 26%, 16.5% and 9.5%, respectively. Relative abundance of *Lachnospiraceae*

Table 2 Linear mixed effects models investigating the effect of host strain and *H. polygyrus* infective dose on β -diversity of the fecal microbiota. Dissimilarity was assessed using two metrics: Bray-Curtis and unweighted UniFrac distances. For each metric, we ran two models, the full model and the model with no host strain \times infective dose interaction. To estimate the host strain effect, the SJL mouse strain was set as reference. Each model also included mouse ID as a random factor. The marginal R^2 was 52.8% and 60.5% for Bray-Curtis and unweighted UniFrac distances, respectively

Model	Type 3 tests of fixed effects			Type 3 tests of fixed effects		
	Estimate (SE)	F	P	Estimate (SE)	F	P
Bray-Curtis distance						
Model with interaction						
Intercept	0.474 (0.025)			0.415 (0.009)		
Host strain (BALB/c)	-0.058 (0.030)	2.72	0.0688	-0.025 (0.011)	Host strain	3.27
Host strain (CBA)	-0.072 (0.032)			-0.029 (0.012)	Infective dose	2, 47.5
Infective dose	0.0004 (0.00006)	113.26	<0.0001	0.0001 (0.00002)	Host strain \times Infective dose	1, 45.1
Host strain (BALB/c) \times infective dose	-0.00001 (0.000008)	1.22	0.2976	0.00002 (0.00003)	Host strain \times Infective dose	2, 43.1
Host strain (CBA) \times infective dose	-0.0001 (0.000008)			-0.00004 (0.00003)		
Unweighted UniFrac distance						
Model with no interaction						
Intercept	0.487 (0.016)			0.415 (0.006)		
Host strain (BALB/c)	-0.063 (0.016)	19.12	<0.0001	-0.018 (0.006)	Host strain	17.78
Host strain (CBA)	-0.108 (0.017)			-0.040 (0.007)	Infective dose	2, 45.7
Infective dose	0.0003 (0.00003)	128.55	<0.0001	0.0001 (0.00001)	Infective dose	1, 41.4

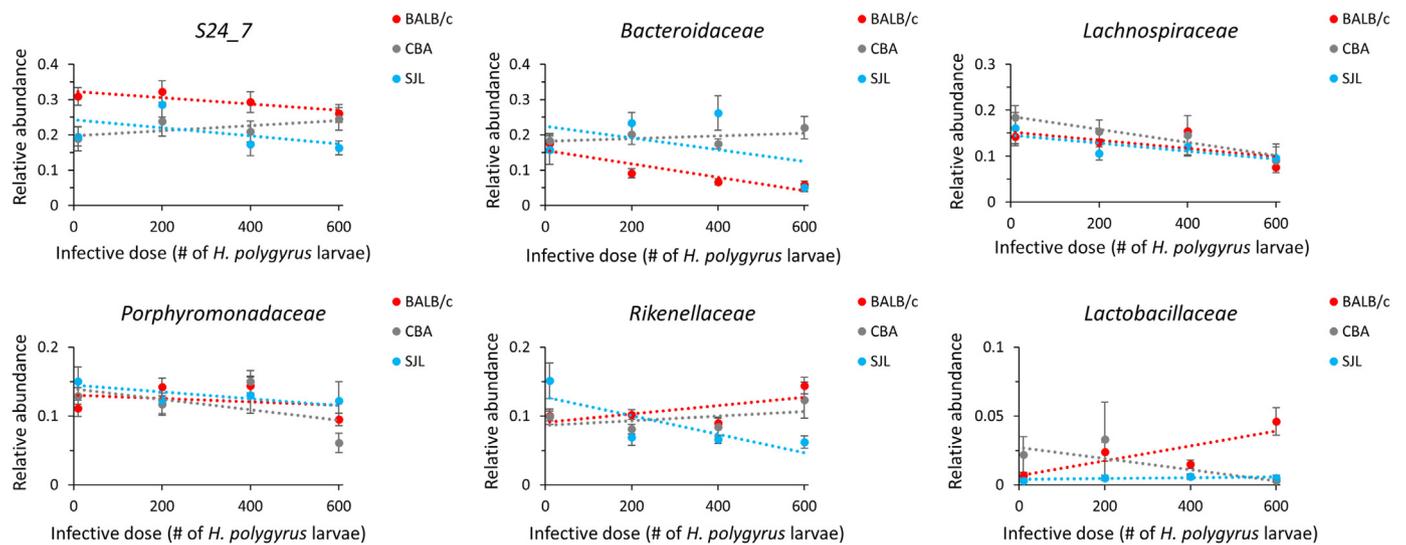


Fig. 2. Changes in relative abundance of the five commonest bacterial families, and *Lactobacillaceae*, across infective doses (number of *Heligmosomoides polygyrus* larvae) in three mouse strains. We report means \pm standard errors. Dotted lines refer to the fit of the linear mixed effects models.

declined with infection burden (26.8% of variance explained by infective dose (Fig. 2; Table 3; Supplementary Table S2). Relative abundance of S24-7 varied among host strains [21.1% of variance explained (Fig. 2; Table 3; Supplementary Table S1; Supplementary Table S2)]. Finally, relative abundance of *Porphyromonadaceae* varied independently from both host strain and infective dose (less than 10% of variance explained by each term) (Fig. 2; Table 3; Supplementary Table S2).

4. Discussion

We investigated the reaction norms of the diversity and composition of the fecal microbiota along a gradient of infection with a gastrointestinal nematode in three host strains. We found that α -diversity (OTU richness) declined with infective dose and differed among host strains (both OTU richness and Shannon diversity). Similarly, β -diversity increased along the gradient of infection burden (indicating more dissimilar microbiota composition between uninfected and infected mice) and varied among host strains. However, the rate of change of α - and β -diversity was similar for the three host strains, as indicated by the non-significant interaction between host strain and infective dose. Focusing on the main bacterial families that occur in the gut, we found evidence for a $G \times E$ interaction for three of them (*Rikenellaceae*, *Lactobacillaceae* and *Bacteroidaceae*).

Although the effect of host genetic background and environmental variation on the diversity and composition of the gut microbiota has attracted considerable attention (see Spor et al. (2011) for a review), to the best of our knowledge no studies have explicitly addressed whether the effect of helminth infection is similar across different host genetic backgrounds. Evidence suggesting that the diversity and composition of the microbiota respond to environmental conditions depending on the phenotype of the host comes from studies that have reported differential effect of diet composition between males and females. For instance, high-fat diet has recently been shown to alter the gut microbiota of zebrafish (*Danio rerio*) differently in males and females (Navarro-Barron et al., 2019), and similar results have also been reported in mice (Han et al., 2018). Whether these phenotype \times environment interactions reflect genotype \times environment interactions is an open question.

We used three mouse strains that have different patterns of resistance to *H. polygyrus*, and different expression of local (intestinal) immune effectors (innate and acquired, humoral and cellular) (Filbey et al., 2014). The choice of the different host genetic backgrounds was therefore based on the assumption that by producing different immune responses to the nematode (both in terms of type and strength of the response), the

different host strains would also provide changing environmental conditions likely to affect the diversity and composition of the microbiota.

If different genotypes respond differently to varying levels of helminth infection in terms of the diversity and composition of the gut microbiota, this can potentially have several ecological and evolutionary consequences. Helminths are very prevalent parasites in wildlife, domestic animals and humans living in developing countries. In humans, health-debilitating effects of soil-transmitted helminth infection depend on the parasite burden and range from asymptomatic (low burden) to impaired growth and development (high burden) (WHO, 2018). In livestock, intestinal worms have been recognized since long time as a factor impairing growth and other traits related to performance (Forbes et al., 2000). Finally, in wildlife, helminths have been reported to have debilitating effects on host fitness-linked traits, such as reproductive output and survival (Pedersen and Greives, 2008). Therefore, helminths do incur fitness costs to their hosts. We suggest that one component of such costs might come from an altered gut microbiota. If, as reported here, high levels of helminth infection are associated with changes in microbiota diversity and composition, impaired host health might indirectly arise through dysbiosis. However, if different host genotypes manage to maintain a diverse microbiota, with key bacterial taxa, in the face of infection, they might be able to pay a lower cost (e.g. maintain a better health status) compared to genotypes whose microbiota is more substantially altered by the infection. In other terms, we suggest that the host genotype-specific capacity to maintain a stable and diverse microbiota might be seen as a tolerance mechanism (Medzhitov et al., 2012). In addition to the direct effect of infection, due to the consumption of resources that are no longer available for the host, helminths can also impinge on host health by facilitating the infection by other parasites (Ezenwa, 2016). While it has been often suggested that the positive association between helminth and microparasite infection is due to the immunosuppressive effect of helminths (Maizels et al., 2004), recent work has shown that the microbiota can also play such a role. Mice infected with *H. polygyrus* are more prone to suffer from severe infection from the intestinal parasite *Citrobacter rodentium* (Chen et al., 2005), and, with a series of elegant experiments, Su et al. (2018) showed that the cause of such increased susceptibility to *C. rodentium* was due to an altered microbiota. If these results can be extrapolated to a natural context, one might predict that host genotypes able to maintain a steady microbiota might be more resistant to other opportunistic diseases. Therefore, $G \times E$ interactions in the diversity and composition of the microbiota might play a role in the maintenance of genetic diversity of host tolerance and resistance.

Table 3 Linear mixed effects models investigating the effect of host strain and *H. polygyrus* infective dose on relative abundance of five bacterial families with abundance > 10% and *Lactobacillaceae*. For each bacterial family, we ran two models, the full model and the model with no host strain \times infective dose interaction, with the exception of *Rikenellaceae*, *Lactobacillaceae* and *Bacteroidaceae*, for which the host strain \times infective dose was statistically significant. Each model also included mouse ID as a random factor. The marginal R^2 was 13.3%, 14%, 5.1%, 13.6%, 26.1% and 27.4% for S24-7, *Lachnospiraceae*, *Porphyromonadaceae*, *Rikenellaceae*, *Lactobacillaceae* and *Bacteroidaceae*, respectively

Bacterial family	S24-7			<i>Lachnospiraceae</i>			<i>Porphyromonadaceae</i>			<i>Rikenellaceae</i>			<i>Lactobacillaceae</i>			<i>Bacteroidaceae</i>		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Model with interaction																		
Host strain	6.58	2, 161	0.002	0.92	2, 57.6	0.403	0.22	2, 52.3	0.803	2.59	2, 51.7	0.084	2.32	2, 52.3	0.109	2.08	2, 45.6	0.008
Infective dose	1.07	1, 161	0.303	8.66	1, 53.3	0.005	3.75	1, 48.9	0.059	0.45	1, 48	0.506	0.19	1, 48.2	0.664	7.72	1, 42.4	0.137
Host strain \times Infective dose	1.77	2, 161	0.173	0.27	2, 50.7	0.763	0.40	2, 47.2	0.674	8.66	2, 46	0.001	5.42	2, 45.6	0.008	3.74	2, 40.8	0.032
Model with no interaction																		
Host strain	10.22	2, 163	<0.001	0.86	2, 55.7	0.430	0.37	2, 52.6	0.690									
Infective dose	1.29	1, 163	0.259	9.43	1, 48	0.004	3.46	1, 47.1	0.069									

Investigating $G \times E$ requires large sample size, which might come at odds with the ethical requirements to keep the number of animals for research purposes at its minimum. To address how much the sample size that was actually available affected the probability to detect statistically significant $G \times E$ interactions, we performed a retrospective power analysis. This analysis showed that our sample size was large enough to detect $G \times E$ interactions, provided the reaction norms had different signs (positive and negative slopes relating α - or β -diversity and infective dose in the three host strains). Therefore, we can safely conclude that we did not find any evidence for strong $G \times E$ interactions. Similarly, the simulations suggested that even with very large sample size (up to four times the available one), we might not have had enough power to detect significant $G \times E$ interactions, again suggesting that the evidence in support to $G \times E$ was at best weak. A different picture emerged when focusing on the relative abundance of the commonest bacterial families and on *Lactobacillaceae*, because in three out of six cases we found support for $G \times E$ interactions, with relative abundance increasing or decreasing as infection burden increased in the three host strains. The discrepancy between the results on α -diversity, β -diversity and relative abundance of bacterial families might be due to the fact that the metrics used to describe diversity and dissimilarity integrate the whole bacterial community and therefore might be less sensitive than the relative abundance of specific bacterial taxa. We would also stress that a possible caveat of the analyses on relative abundances is that these values are not independent. Therefore, in principle, any effect of a given environmental factor (e.g. the infection burden) on the abundance of one bacterial family might be mirrored by the opposing effect on the abundance of the other families. However, we suggest that this is more likely to occur when higher taxonomic groups are considered, such as phyla, where changes in the abundance of one phylum (e.g. Firmicutes) are directly mirrored in the abundance of the other main phylum (e.g. Bacteroidetes).

The finding of crossing reaction norms for *Lactobacillaceae* abundance is particularly interesting in the light of previous reports suggesting that the abundance of *Lactobacillus* sp. might be an important factor contributing to the susceptibility to *H. polygyrus* (Walk et al., 2010; Reynolds et al., 2014; Su et al., 2018). Our results suggest that there might be no such a general link between infection with *H. polygyrus* and *Lactobacillus* abundance. Therefore, as mentioned above, this result suggests that *Lactobacilli* might contribute to shape resistance or tolerance to *H. polygyrus* infection in a host genotype-specific way. The comparison of our results with those reported by other groups, actually suggests that in addition to host genetic background, the original composition of the microbiota might also play a role. Indeed, for the same host strain (BALB/c) different effects of *H. polygyrus* infection on *Lactobacillus* abundance have been reported (Reynolds et al., 2014; Su et al., 2018; this study); although it should also be acknowledged that these previous studies used different protocols (fecal vs ileum or cecum microbiota; single infective dose vs multiple doses). By the way, when comparing the change in relative abundance in *Lactobacillaceae* in BALB/c mice between controls and individuals infected with 200 *H. polygyrus* larvae (the dose used in previously published work), we did not find any difference ($t = -1.66$, $P = 0.1387$, $n = 10$ mice and 38 observations). The fact that the same mouse strain coming from different vendors has different microbiota composition has already been reported (Villarino et al., 2016). Therefore, the trajectories linking infection burden and the abundance of specific bacterial taxa might actually depend on both the host genetic background and the environmental-dependent initial composition of the microbiota. If this applies to a natural context, it might actually produce a mosaic of populations varying in infection burden, microbiota diversity and composition, and host genotype frequency that might also contribute to the process of local adaptation.

In addition to the issue concerning the statistical power associated with our sample size, another possible concern is related to the choice of using three inbred strains of laboratory mice, which obviously do not

represent the amount of genetic variation present in natural rodent populations. With this respect, it is also important to stress that diversity and composition of the gut microbiota differs between wild house mice and laboratory strains (Rosshart et al., 2017). Therefore, we cannot exclude that in wild mice the natural microbiota might be more resilient to the perturbation induced by a high infection burden, or that evidence in support to G × E interactions might have been stronger when using a larger range of genotypes derived from natural populations. Using natural populations of *H. polygyrus*-infected mice should also help to better identify the role of key bacterial taxa (such as *Lactobacilli*) as a tolerance mechanism, and how it varies among genotypes within and between populations of hosts.

5. Conclusions

To conclude, our study provides evidence showing that infection with the gastrointestinal nematode *H. polygyrus* depauperates the diversity of the intestinal microbiota (α -diversity) and alters its composition (β -diversity) in a dose-dependent manner. We investigated these effects in three mouse genotypes to assess the possible G × E interactions, but found, at best, weak evidence in support for them. Focusing on specific bacterial families provided, nevertheless, evidence for G × E interactions, suggesting that the outcome of the host-parasite interaction might be determined by keystone bacterial taxa whose action might depend on the host genetic background.

Funding

Funding was provided by the French Agence Nationale de la Recherche (projects EVOREGIM and COINFECTION).

Ethical approval

All animal experiments were approved by the Comité d'Éthique de l'Expérimentation Animale Grand Campus Dijon, France (CNREEA n° C2EA-105) and by the Ministère de la Recherche et de l'Enseignement Supérieur (project # 01867.01).

CRedit author statement

Emmanuel Guivier: conceptualization, methodology, data curation and analysis, writing - reviewing and editing. Maxime Galan: methodology, microbiota analysis, data curation, writing - reviewing and editing. Cédric Lippens: methodology, data curation, writing - reviewing and editing. Jérôme Bellenger: methodology, writing - reviewing and editing. Bruno Faivre: conceptualization, methodology, data curation, writing - reviewing and editing. Gabriele Sorci: conceptualization, methodology, data curation and analysis, writing - original draft preparation, writing - reviewing and editing. All authors read and approved the final manuscript.

Data availability

The data upon which this article is based are available in [Supplementary file 2](#).

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are very grateful to Valerie Saint-Giorgio and all the staff of the animal facility, Université de Bourgogne, France.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crpvbd.2022.100082>.

References

- Belkaid, Y., Hand, T.W., 2014. Role of the microbiota in immunity and inflammation. *Cell* 157, 121–141.
- Cattadori, I.M., Sebastian, A., Hao, H., Katani, R., Albert, I., Eilertson, K.E., et al., 2016. Impact of helminth infections and nutritional constraints on the small intestine microbiota. *PLoS One* 11, e0159770.
- Chen, C.C., Louie, S., McCormick, B., Walker, W., Shi, H., 2005. Concurrent infection with the intestinal helminth parasite impairs host resistance to enteric *Citrobacter rodentium* and enhances *Citrobacter*-induced colitis in mice. *Infect. Immun.* 73, 5468–5481.
- Ezenwa, V.O., 2016. Helminth-microparasite co-infection in wildlife: lessons from ruminants, rodents and rabbits. *Parasite Immunol.* 38, 527–534.
- Faivre, B., Bellenger, J., Rieu, A., Guivier, E., Galan, M., Ollivier, A., et al., 2019. Disentangling the effect of the host genetics and gut microbiota on resistance to an intestinal parasite. *Int. J. Parasitol.* 49, 873–883.
- Filbey, K.J., Grainger, J.R., Smith, K.A., Boon, L., van Rooijen, N., Harcus, Y., et al., 2014. Innate and adaptive type 2 immune cell responses in genetically controlled resistance to intestinal helminth infection. *Immunol. Cell Biol.* 92, 436–448.
- Forbes, A.B., Huckle, C.A., Gibb, M.J., Rook, A.J., Nuthall, R., 2000. Evaluation of the effects of nematode parasitism on grazing behavior, herbage intake and growth in young grazing cattle. *Vet. Parasitol.* 90, 111–118.
- Galan, M., Razzauti, M., Bard, E., Bernard, M., Brouat, C., Charbonnel, N., et al., 2016. 16S rRNA amplicon sequencing for epidemiological surveys of bacteria in wildlife. *mSystems* 1, e00032-16.
- Gause, W.C., Maizels, R.M., 2016. Microbiota - helminths as active participants and partners of the microbiota in host intestinal homeostasis. *Curr. Opin. Microbiol.* 32, 14–18.
- Glendinning, L., Nausch, N., Free, A., Taylor, D.W., Mutapi, F., 2014. The microbiota and helminths: sharing the same niche in the human host. *Parasitology* 141, 1255–1271.
- Han, J., Cui, C., Li, Y., Gao, H., Zhang, H., Zhang, C., et al., 2018. Dietary supplement with a mixture of fish oil and krill oil has sex-dependent effect on obese mice gut microbiota. *J. Funct. Foods* 51, 47–54.
- Holm, J.B., Sorobetea, D., Küllerich, P., Ramayo-Caldas, Y., Estellé, J., Ma, T., et al., 2015. Chronic *Trichuris muris* infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of *Lactobacilli*. *PLoS One* 10, e0125495.
- Houlden, A., Hayes, K.S., Bancroft, A.J., Worthington, J.J., Wang, P., Grencis, R.K., et al., 2015. Chronic *Trichuris muris* infection in C57BL/6 mice causes significant changes in host microbiota and metabolome: Effects reversed by pathogen clearance. *PLoS One* 10, e0125945.
- Jenkins, T.P., Formenti, F., Castro, C., Piubelli, C., Perandin, F., Buonfrate, D., et al., 2018a. A comprehensive analysis of the faecal microbiome and metabolome of *Strongyloides stercoralis* infected volunteers from a non-endemic area. *Sci. Rep.* 8, 15651.
- Jenkins, T.P., Peachey, L.E., Ajami, N.J., MacDonald, A.S., Hsieh, M.H., Brindley, P.J., et al., 2018b. *Schistosoma mansoni* infection is associated with quantitative and qualitative modifications of the mammalian intestinal microbiota. *Sci. Rep.* 8, 12072.
- Jenkins, T.P., Rathnayaka, Y., Perera, P.K., Peachey, L.E., Nolan, M.J., Krause, L., et al., 2017. Infections by human gastrointestinal helminths are associated with changes in faecal microbiota diversity and composition. *PLoS One* 12, e0184719.
- Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* 79, 5112–5120.
- Kreisinger, J., Bastien, G., Hauffe, H.C., Marchesi, J., Perkins, S.E., 2015. Interactions between multiple helminths and the gut microbiota in wild rodents. *Phil. Trans. R. Soc. B* 370, 20140295.
- LeChatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., et al., 2013. Richness of human gut microbiome correlates with metabolic markers. *Nature* 500, 541–546.
- Lee, S.C., Tang, M.S., Lim, Y.A.L., Choy, S.H., Kurtz, Z.D., Cox, L.M., et al., 2014. Helminth colonization is associated with increased diversity of the gut microbiota. *PLoS Negl. Trop. Dis.* 8, e2880.
- Lippens, C., Guivier, E., Faivre, B., Sorci, G., 2016. Reaction norms of host immunity, host fitness and parasite performance in a mouse-intestinal nematode interaction. *Int. J. Parasitol.* 46, 133–140.
- Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., Knight, R., 2012. Diversity, stability and resilience of the human gut microbiota. *Nature* 489, 220–230.
- Maizels, R.M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M.D., Allen, J.E., 2004. Helminth parasites - masters of regulation. *Immunol. Rev.* 201, 89–116.
- Martin, I., Djuradi, Y., Sartono, E., Rosa, B.A., Supali, T., Mitreva, M., et al., 2018. Dynamic changes in human-gut microbiome in relation to a placebo-controlled anthelmintic trial in Indonesia. *PLoS Negl. Trop. Dis.* 12, e0006620.
- Medzhitov, R., Schneider, D.S., Soares, M.P., 2012. Disease tolerance as a defense strategy. *Science* 335, 969–971.
- Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* 4, 133–142.
- Navarro-Barron, E., Hernandez, C., Llera-Herrera, R., Garcia-Gasca, A., Gomez-Gil, B., 2019. Overfeeding a high-fat diet promotes sex-specific alterations on the gut microbiota of zebrafish. *Zebrafish* 16, 268–279.

- Pedersen, A.B., Greives, T.J., 2008. The interaction of parasites and resources cause crashes in a wild mouse population. *J. Anim. Ecol.* 77, 370–377.
- Reynolds, L.A., Smith, K.A., Filbey, K.J., Harcus, Y., Hewitson, J.P., Redpath, S.A., et al., 2014. Commensal-pathogen interactions in the intestinal tract: *Lactobacilli* promote infection with, and are promoted by, helminth parasites. *Gut Microb.* 5, 522–532.
- Rosa, B.A., Supali, T., Gankpala, L., Djuardi, Y., Sartono, E., Zhou, Y., et al., 2018. Differential human gut microbiome assemblages during soil-transmitted helminth infections in Indonesia and Liberia. *Microbiome* 6, 33.
- Rosshart, S.P., Vassallo, B.G., Angeletti, D., Hutchinson, D.S., Morgan, A.P., Takeda, K., et al., 2017. Wild mouse gut microbiota promotes host fitness and improves disease resistance. *Cell* 171, 1015–1028.
- Schneeberger, P.H.H., Coulibaly, J.T., Panic, G., Daubenberger, C., Gueuning, M., Frey, J.E., et al., 2018. Investigations on the interplays between *Schistosoma mansoni*, praziquantel and the gut microbiome. *Parasit. Vectors* 11, 168.
- Spor, A., Koren, O., Ley, R., 2011. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat. Rev. Microbiol.* 9, 279–290.
- Su, C., Su, L., Li, Y., Long, S.R., Chang, J., Zhang, W., et al., 2018. Helminth-induced alterations of the gut microbiota exacerbate bacterial colitis. *Mucosal Immunol.* 11, 144–157.
- The Human Microbiome Project Consortium, 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207–214.
- Villarino, N.F., LeCleir, G.R., Denny, J.E., Dearth, S.P., Harding, C.L., Sloan, S.S., et al., 2016. Composition of the gut microbiota modulates the severity of malaria. *Proc. Natl. Acad. Sci. U.S.A.* 113, 2235–2240.
- Walk, S.T., Blum, A.M., Ewing, S.A., Weinstock, J.V., Young, V.B., 2010. Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. *Inflamm. Bowel Dis.* 16, 1841–1849.
- Wegener Parfrey, L., Jirku, M., Sima, R., Jalovecka, M., Sak, B., Grigore, K., et al., 2017. A benign helminth alters the host immune system and the gut microbiota in a rat model system. *PLoS One* 12, e0182205.
- WHO, 2018. Soil-transmitted Helminth Infection. <https://www.who.int/en/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>. (Accessed 7 October 2019).
- Yang, C.A., Liang, C., Lin, C.L., Hsiao, C.T., Peng, C.T., Lin, H.C., et al., 2017. Impact of *Enterobius vermicularis* infection and mebendazole treatment on intestinal microbiota and host immune response. *PLoS Negl. Trop. Dis.* 11, e0005963.
- Zaiss, M.M., Harris, N.L., 2016. Interactions between the intestinal microbiome and helminth parasites. *Parasite Immunol.* 38, 5–11.